Vol. 11 No. 5

May, 1945

THE BOTANICAL REVIEW

Interpreting Botanical Progress

Decennial Supplementary Review Issue No. I

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PROPOSED FUTURE CONTENTS ON REAR COVER

Published Monthly except August and September at
North Queen St. and McGovern Ave., Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at Lancaster, Pennsylvania, under the Act of March 3, 1879.

THE BOTANICAL REVIEW

Founded in 1935 by
H. A. GLEASON AND E. H. FULLING
Managed, edited and published at
The New York Botanical Garden
by
E. H. FULLING

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THE BOTANICAL REVIEW is published monthly except August and September. Subscription rate for the United States is $4.00 per year; for Canada and other foreign countries $4.25; single copies, 50¢. All subscriptions are payable in advance at par in Lancaster, Pa., or New York. Checks should be made payable to THE BOTANICAL REVIEW.

Manuscripts are prepared by contributing authors only upon invitation and on the suggestion or with the approval of the advisory editor in whose field the subject lies. Unsolicited manuscripts will not be considered. The editor, nevertheless, welcomes suggestions of topics which might properly be reviewed.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at N. Queen St. and McGovern Ave., Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XI MAY, 1945 No. 5

THE STRUCTURE OF PROTOPLASM. II[1]

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INTRODUCTION

Ten years have elapsed since the structure of protoplasm was reviewed in this journal. Five of these years have been trying ones for those foreign countries which in the past have contributed so much to theoretical research. The paucity in original theoretical work has, however, been compensated for by numerous interesting compendia of research in physical and chemical biology. These surveys have appeared as textbooks, monographs, symposia, handbooks and reports in biology and medicine. Such publications are in themselves reviews, but they include new and original work, especially in the realm of interpretation. Among them are the large collection of papers entitled "Medical Physics", edited by Glasser (22); the publication in honor of Professor Bensley, edited by Hoerr (25); the symposium on "The Cell and Protoplasm", held at Leland Stanford University and edited by Taylor (79); the text by Pfeiffer (59) on "Experimentelle Cytologie"; Guilliermond's (24) "Cytoplasm of the Plant Cell"; the fifth volume of "Colloid Chemistry, Theory and Methods, Biology and Medicine", edited by J. Alexander (1); and the symposium on "The Structure of Protoplasm", edited by Seifriz (71).

Should we carry the subject of this review to its logical conclusion, it would include everything that goes on in protoplasm, for in the last analysis structure underlies all natural phenomena. It would seem, for example, that photosynthesis and respiration could be discussed to the fullest extent without mention of structure, yet we could hardly escape a consideration of molecular structure, and if we stop short of it, this is only because of the limitations of our knowledge. Photosynthesis and respiration will not be considered

[1] Supplement to article in The Botanical Review 1: 18–36. 1935.

in this review, but such protoplasmic properties as permeability, anesthesia and frost resistance will be, for certain hypotheses of these phenomena are in learge measure based on structure.

There are a number of properties of protoplasm which appear to have little to do with structure, though actually very closely associated with it and often dependent upon it. Sometimes they are the best indications that we have of a specific type of structure, a structure which is not visible and which therefore must be interpreted on the basis of known properties. As an illustration, an elastic soap may be compared with an inelastic one. Familiarity with jellies and pastes leads to the obvious conclusion that the structural units of the elastic soap are of such a form and arrangement as to endow the dispersion with elastic properties, whereas neither the shape nor orientation of the structural units of the inelastic paste contributes to elastic qualities.

Those properties of protoplasm which are related to structure shall first hold our attention.

PROPERTIES RELATED TO STRUCTURE

Viscosity. Viscosity, in the pure and strict sense, has far less bearing on structure than anomalous viscosity, or so-called non-Newtonian behavior which is a kind of lawless flow. But before taking up anomalous viscosity something should be said on the misuse which has been made of viscosity in interpreting protoplasmic behavior.

Changes in viscosity, and in surface tension and permeability as well, though significant properties of living matter which come into play in many vital processes, are not always, if indeed usually, the cause of the phenomena which they accompany; they are more often the result of, or incidental to the processes, of which they are sometimes said to be the cause. To cite an example: when protoplasmic flow is diminished or stopped by an experimental condition, the assumption is frequently made that an increase in viscosity has occurred and that this increase is responsible for the decreased flow. The rate of protoplasmic movement depends upon two major factors, viscosity and motive force. One or the other or both may be responsible for a change in rate of streaming. The situation may be compared to water flowing through a pipe under pressure exerted by a pump. If the pump breaks down and flow stops, the

viscosity of the water has not therefore increased. Kamiya (26) has found it possible to stop protoplasmic streaming by mechanical pressure without any change in viscosity occurring. Similarly have surface tension and permeability been resorted to in explanation of changes in cellular activities. They are undoubtedly involved and may at times be the cause, but they are just as likely to be among the consequences. If a cell is in a pathological condition from any cause, its control over selective permeability is probably lost to some degree, but the change in permeability is not therefore the cause of the pathological condition. One may make another analogy: diabetic patients have acid blood, but diabetes is due to a breaking down in sugar metabolism and not to the loss of acidity control.

Viscosity changes are significant in cellular physiology. They are best described as diagnostic symptoms. They are sometimes closely correlated with structural changes, as in thixotropic setting and collapse; but it is the anomalous flow of liquids which so closely bears on structure and is so important an indicator of a specific type of structure.

Anomalous Viscosity. When a pure liquid, such as water or glycerine, or a true solution flows it obeys Newton's law, $v = \phi Fr$, in which v is the velocity of flow, ϕ the fluidity, and F the shearing stress on either of two planes separated from each other by the distance r. If the flow fails to follow Newton's law, then something can be said about structure. It is theoretically to be expected and experimentally capable of demonstration that non-Newtonian substances, those which in solution do not exhibit true viscous flow and therefore do not follow Newton's law nor the formula of Poiseuille, possess structural features not present in Newtonian substances. The best illustration of this is that of two soaps, presumably of identical chemical constitution, both sodium stearate, but one Newtonian and the other not. Colloidal dispersions of these soaps, apparently identical, were not so in physical qualities; one exhibited true viscous flow whereas the other showed variable viscosity, the viscosity value varying with each pressure applied. The former Newtonian soap had a granular amorphous structure, microscopically viewed; the latter was crystalline in nature, its dispersed particles were slender fibers, hence the anomalous behavior. Furthermore, the anomalous soap was elastic and the Newtonian soap inelastic.

The distinguishing features of non-Newtonian solutions are two: with each change in pressure they yield a different viscosity value until a definite maximum pressure is reached, at which point the viscosity value remains constant; and they require an initial pressure to start flow; this pressure is termed the yield value. The Society of Rheology owes its origin to these misbehaving non-Newtonian solutions (12). Out of that Society much good has come, but with it one confused issue, the introduction of the term "plastic" to indicate the general character of non-Newtonian substances. This is an unfortunate choice of words, for plastic substances maintain any form into which they are molded, whereas elastic substances do not, and non-Newtonian substances are elastic. Putty is plastic, gelatine not, and gelatine is elastic; solutions of gelatine are non-Newtonian.

Experimental proof of the non-Newtonian character of protoplasm is very meager. A decade ago one could have questioned it, but on experimental grounds only, not on theoretical grounds. Now, however, owing to the work of Pfeiffer (58), it can be conclusively stated that what was before theoretically evident, is now experimentally proved. Pfeiffer has shown that if protoplasts are deformed under different pressures, curves showing pronounced anomalous viscosity are obtained. The flow of protoplasm is therefore not obedient to the laws of Newton, Poiseuille, and Stokes. That protoplasm belongs to the large group of organic substances which are non-Newtonian in behavior has been repeatedly emphasized by Seifriz (66). Needham *et al.* (48) use anomalous viscosity and related properties as a basis for classification of proteins.

Northen and his collaborators (52–55) have carried out a series of studies on structural viscosity which indicate the bearing of non-Newtonian qualities on protoplasmic structure. In order to explain some features of stimulation, Northen (52) advanced the hypothesis that stimulation breaks down the protoplasmic network. He adds that many features of irritability may be explained if protoplasm is considered a reversibly dissociable system. Northen and MacVicar (55) found that when filaments of *Spirogyra* are subjected to X-ray dosages from 250 to 5000 r units, the structural viscosity at first decreases, and then, with longer exposure, increases. Decrease in structural viscosity may indicate that constituents of the protoplasmic network have dissociated or that long protein mole-

cules have shortened, whereas increases in viscosity would mean that molecules have extended or that reassociations have occurred. Muir (45) applies the same interpretations to changes in viscosity due to salts and oleates. He says that decrease in structural viscosity brought about by these substances may be the result of a weakening or destruction of bonds such as those existing between the lipophilic side chains of protein molecules. Northen (52) interprets the anesthetic effects of fat-solvents in terms of readjustments in protoplasmic structure.

Double Refraction. Many of the physical properties of protoplasm are so closely correlated, so intimately interdependent, that to mention one is to imply the others. This is true of the relationship between the non-Newtonian behavior of protoplasm, its double refraction or flow birefringence, fluid crystallinity, elasticity, contractility, and continuity in structure. The double refraction of protoplasm has been frequently denied because it is not readily demonstrable; actually, however, its presence has long been known. Englemann (19), as far back as 1875, observed it in pseudopodia; later Weber (81) described it in chloroplasts, which, though not of protoplasm, are closely akin to it. And, finally, Schmidt (64) has described double refraction in *Amoeba.*

Flow Birefringence. Birefringence is synonymous with double refraction, but as flow birefringence it becomes a special case particularly applicable to protoplasm, for the living substance is often in a state of flow. Birefringence in a flowing liquid may be seen in certain colloidal suspensions such as that of vanadium pentoxide, but only when these are in a state of flow. This behavior means that linear particles, present in the liquid, are scattered in a disorderly fashion when the liquid is in a state of rest, but during flow they become oriented, and double refraction or flow birefringence results.

Flow birefringence is becoming a commonplace in biological literature. Some years ago the reviewer insisted that protoplasm must and would show double refraction if proper tests could be made. Up to that time only the forgotten work of Engelmann (19) existed in support of it in protoplasm. Later came the direct work of Schmidt (64) and the indirect evidence of Pfeiffer (58). Both indicate that fibril molecules are present in protoplasm, that these are the cause of anomalous viscosity and birefringence.

Great emphasis on birefringence in organic matter has been laid

by Needham and his co-workers (17, 29, 30, 46, 47) in their study of protein solutions. Their problem was a combined study of anomalous viscosity and flow-birefringence. Joining these two phenomena in one study indicates appreciation of their close relationship and the interdependence of all properties based on a specific type of structure. As a result of their experiments on protein solutions, both in bulk and as surface films, Needham and co-workers (29, 30) class proteins into three major groups. The first group consists of those proteins which show flow-anomaly, *i.e.*, are non-Newtonian, both in the bulk phase and in the surface film. They also show flow-birefringence in the bulk phase. Examples are tobacco mosaic virus, nucleoprotein and myosin. Though corpuscular proteins, they have elongated particles before denaturation. The second group consists of those proteins which show flow-anomaly only in the surface film and no flow-birefringence in the bulk phase. They are probably close to spherical in shape in solution, but form elongated particles as they denature in the surface film. After this process has been completed, they may show flow-anomaly also in the bulk phase. An example is serum euglobulin. The third group consists of those proteins which show flow-anomaly neither in the bulk phase nor in the surface film. They are probably close to spherical in shape. Examples are insulin and methaemoglobin. In their third paper (17) the authors emphasize the theoretical significance of protein fiber molecules in relation to experimental morphology and cytology.

Elasticity. The importance of elasticity in protoplasmic studies has long been emphasized by Scarth (61) and Seifriz (65). In Scarth's words, "elasticity is the chief quality of protoplasm on which a conjecture of the ultra-microscopic structure of living matter can be based".

In his studies on protoplasmic elasticity, Norris (49) made a determination of Young's modulus for a strand of myxomycete plasmodium. The amount of bending of a calibrated needle with which the protoplasm is being stretched is a measure of the force exerted. He found the modulus of elasticity for strands of *Physarum* to be 9.0×10^4 dynes per cm.^{2} at room temperature for increases in length up to 40%. When the temperature was lowered to 10° C., there was an increase in the average value of Young's modulus to over twice that found at room temperature; the strands

no longer followed Hooke's law. With increases in temperature the value of Young's modulus was lowered.

A very important observation by Pease (57), one answering a long controversy, was that bearing on the place where the material responsible for the elasticity of protoplasm is situated; it is not concentrated at the surface, as shown by tests with saponin. Pease noted also that chloretone anesthesia has no effect on the elasticity of *Physarum* strands, although streaming is reversibly stopped.

Contractility. Contractility is closely related to elasticity; to it Seifriz (66) attaches much importance, particularly rhythmic contractility (68) which he regards as a potential property of all protoplasm, manifesting itself only in tissue where it is needed, as in heart muscle, intestines, etc. Contractility is to be seen in many forms of cellular activity, in the migration of fibroblasts, in protozoan flagellae, and in myxomycetes. Seifriz (67) has based a theory of protoplasmic streaming on the rhythmic contractility of protoplasm, on the continuous rhythmic pulsation of the protoplasm of slime molds. He (72) states that if time-lapse moving pictures of the streaming protoplasm of the myxomycete, *Physarum polycephalum,* are made, a remarkable pulsation of the plasmodium is revealed. Pictures taken every five seconds and shown at the usual rate of sixteen per second, which means a speeding up of eighty times, show the plasmodium undergoing rhythmic contractility and relaxation in perfect synchronism with the protoplasmic flow. The rhythmic contractions and expansions when thus optically accelerated resemble the pulsations of a heart. At each contraction and relaxation the direction of protoplasmic flow reverses, and with the outward flow the plasmodium advances slightly.

The mechanism of protoplasmic movement in slime molds is, then, one of rhythmic contraction and relaxation of the plasmodium, with a total of 95 seconds for each pulsation, 45 seconds for systole and 50 seconds for diastole. The additional five seconds in time of outward flow account for advancement. Kamiya (27) gives 93.7 seconds for one rhythmic period.

Seifriz (72) emphasizes the widespread occurrence of rhythmic contractility in animate nature, in muscle, nervous impulses, individual cells, *etc.*

Spirality. The spiral characteristics of living matter have been but little studied, yet some observations and general conclusions have been made.

The reviewer has long laid emphasis on the spiral qualities of living matter, whether a chromosome or tree trunk. It appears that much in nature is asymmetrical, from the carbon atom and crystals to organisms. Growth and movement take a spiral course.

Castle (14), by automatic photographic recording, studied the elongational and rotational, that is to say, spiral components, of the growth of the sporangiosphore of *Phycomyces.*

That protoplasm should exhibit spiral tendencies is obvious from the asymmetry of organisms and the asymmetry of the crystalline matter of which protoplasm is made. The extent to which spiral movement as in protozoa, spiral growth as in horns, tusks and trees, and spiral shapes as in chromosomes, can be attributed to the asymmetry of crystals, or the asymmetry of the carbon atom, or to helical organic molecules, is a matter of conjecture, but spirality in living matter is present, nevertheless, and in many forms.

Torsion. If spirality is a basic quality of protoplasm, then a state of torsion may often if not usually exist in protoplasm. Having reached this likely conclusion, research was set underway to prove it, and with surprising results. As the work done has not yet appeared in print, only a general statement can here be made. During the flow of protoplasm through a single freely suspended strand of the myxomycete, *Physarum,* the strand is twisted first to the right and then to the left as the flow of protoplasm moves first in one and then in the other direction. At each point of maximum twist a state of torsion is set up which must be relieved; consequently, there is a winding and unwinding of the strand. At the moment of each reversal in the winding and the unwinding, the state of torsion is at a maximum. The twisting of the strand is correlated with the flow of the protoplasm. The precise nature of this correlation is a particularly interesting but troublesome question which it is hoped may be answered.

Ductility. Ductility, or the capacity to be drawn out, is characteristic of protoplasm. Ordinarily it is accompanied in protoplasm by a return movement, that is to say, protoplasm is elastic, but ductility as such does not involve elasticity.

Protoplasm is highly ductile (65). If the protoplasm extended is tough, resilient and elastic, it will return with great suddenness when severed from the needle, by means of which it is being stretched. That it clings to the needle, though subjected to a sub-

stantial tension, is evidence of its high tackiness. That it often returns after being stretched is evidence of its elastic qualities, and that it may be drawn to very great lengths is proof of high ductility. The last property is great, perhaps greatest, when the protoplasm is poorly elastic, as Pfeiffer has shown (58, 59).

Tackiness. Whatever controversy may exist on the presence or absence of certain physical properties of living matter, no one has seriously questioned the existence of glutinous or tacky qualities. Protoplasm is adhesive, cells cling together. Did they not, we should, says Lewis (34), fall apart. Now the admission of tackiness carries with it admission of elasticity. These properties, and some others such as surface tension, are often confused with viscosity, it being assumed that high viscosity means high adhesive qualities, high surface tension, *etc.*, but this is not true. There is no correlation between viscosity, on the one hand, and tackiness, surface tension, tensile strength and elasticity, on the other.

The foregoing properties all owe their origin to specific structural features, tactility like elasticity being a particularly strong indication of continuity in structure.

Thixotrophy. The last manuscript written by Professor Freundlich (20) was on thixotrophy. He introduced the subject with these words: "In older textbooks of physiology, let us say of about 1900, the state of aggregation of protoplasm is left very unclear. It is frequently considered to be fluid. On the other hand, it is emphasized that life processes seem to require a well-defined 'organization' and that such an organization can hardly be imagined without some kind of a more or less solid structure. There is practically no indication of the fact that the mechanical properties of protoplasm differ essentially from those of normal liquids or solids and that they may be correlated with its colloidal nature. References to colloids are found, if at all, when the impermeability of membranes to proteins, or swelling, or the scarcity of distinct crystals, are discussed.

"This outlook has completely changed during the last twenty years. It has been found that there may exist a number of intermediate stages between the normal liquid state and the state of a crystalline solid. These are frequently observed in colloidal systems, particularly if they contain a sufficiently high amount of disperse phase. Consequently, concentrated colloidal solutions may differ essentially in their mechanical properties (viscosity, elasticity,

etc.) from normal so-called Newtonian liquids. These differences may be manifold. Two limiting cases are of outstanding importance; the first is that of thixotrophy. . . . The other limiting case is that of dilatancy. . . . Although observed and named by Osborne Reynolds in 1885, dilatancy has only recently been recognized as a remarkable counterpart to thixotrophy. Osborne Reynolds used the term when describing the behavior of moist quartz sand; it whitens and appears to be dry when the foot falls on it and becomes wet again when the foot is raised. An aqueous suspension of finely ground powder (particle size 1 to 5 μ) at a concentration of about 44 per cent of the solid is strongly dilatant. A glass rod can easily be moved through the mass at low speed, but an enormous resistance is set up if the speed is increased above a certain limit. . . . Suspensions of intact starch grains in water are strongly dilatant too. . . . The particles of a dilatant suspension are quite independent of each other; there is not the least indication of coagulation. On the other hand, the particles of a thixotrophic suspension are always found to be coagulated to a certain degree, sticking together and forming clusters. If brought into suspension in the liquid, they may be temporarily separated from each other, but they always unite again to clusters".

The biological applications of thixotrophy are many (20, 66), one of which will be discussed in the last part of this review.

The capacity of a colloidal substance to collapse from mechanical disturbance and reset instantly is dependent on specific structural characteristics. In short, a study of all physical properties of jellies, living and non-living, ultimately leads to structure.

STRUCTURE

All problems having to do with the physical properties of protoplasm lead to structure, for structure determines these properties. Protoplasmic structure is of four categories: gross structure or cellular organization, microscopic structure, ultramicroscopic or colloidal structure, and molecular structure.

Gross Structure. The gross structure of protoplasm is here taken to mean cellular organization. Scarth (62) has handled the coarser structure of protoplasm unusually well, and clarified the confusion which has arisen in the application of term after term to the successive layers of protoplasm. He distinguishes five layers or ori-

ented substances, which, though they apply to the plant cell, may fit the animal cell as well, with the exception of the outermost wall of cellulose in plants. Within the wall there are, in order, the protoplasmic membrane or ectoplast, the plasma gel or cortical endoplasm, the plasmasol or liquid endoplasm, and the inner tonoplast or vacuolar membrane surrounding the vacuolar fluid, through which kinoplasmic strands thread their way, joining the outer membrane to the tonoplast.

The presence of and need for cellular organization has been appreciated by many workers. Cohn (16) arranges the component parts of cells into patterns. Non-polarized organisms have no fixed axis of development. Polarization may be induced in initially unpolarized forms, *e.g.*, Myxomycetes and Aerasieae, by an asymmetric environment. Gause (21) considers the possible application of organization concepts of topology to asymmetric organization of protoplasm.

Skotnicky (75) states that the conducting layer of nerves is a property of the functional organization of protoplasm.

An example of gross structure or cellular organization is given by Seifriz (72) in the slime molds. In the original moving pictures of slime molds taken by Seifriz, three distinct rhythms of contraction were observed. Subsequent work by Kamiya (27) led to the postulation of many centers of contractility, that is to say, slime mold protoplasm is a polyrhythmic system. There are, therefore, many independent centers of activity, each with an individuality all its own. The physiological state of any one center differs from that of adjoining centers at any moment very much as adjoining cells in tissue differ, with this exception, that in a plasmodium the difference is less permanent.

Further indication that a slime mold is an assemblage of individual units is to be had in the habit plasmodia have of breaking up into microscopic bits at the time of sclerotium formation and during the plasmodial stage. At sclerotium formation, the drying plasmodium fragments into many small sections, the whole resembling an assemblage of cells. These subdivisions represent previous centers of activity which were physiologically distinct at the time the sclerotium was formed. It is possible that they are uninucleate masses of protoplasm. The bits which are distinct morphological units in a sclerotium cease to be so in a plasmodium, where, how-

ever, they retain their physiological individuality, one feature of which is the characteristic rhythm each possesses, a rhythm not necessarily in perfect synchronism with that of other units.

Microscopic Structure. The microscopically visible structure of protoplasm has been reviewed many times, the theories are now classical. Little new has been added, chiefly because it is now generally recognized that the visible structure of protoplasm has nothing to do with the basic structure. Thus, the visible granules and globules, which are of many categories with many names, such as vacuoles, vesicles, mitochondria, macrosomes, microsomes, *etc.*, all play important rôles in the life of the cell, but none is concerned in the structural features of protoplasm, none is a building unit, all are but inclusions. Seifriz (66) has pointed out that although the visible emulsion in protoplasm is of importance from the point of view of nutrition, it is in no way responsible for such properties of protoplasm as permeability, imbibition, elasticity or electric conduction, nor can it in any way represent that structure of living matter which distinguishes it from the non-living. The visible structure is not, as has been said in the past, a coarser manifestation of the finer structure which lies hidden from view. A denial of this does not imply that the visible structure should be ignored, for it plays a significant rôle in the metabolism of life, just as does the still coarser organization of the cell as a whole.

Ultramicroscopic Structure. No hypothesis of protoplasmic structure has been so troublesome as that designating living matter an "emulsoid". Just what an emulsoid is no one seems to know. Gelatine is called an emulsoid, but the word means "like an emulsion", and gelatine is not like an emulsion. The emulsoid hypothesis of gel structure long prevailed in biology because of support from some chemists, among whom was Gortner (23), whose book, excellent in parts, was accepted in toto by some few chemists and biologists. Gortner, in his classification of colloidal systems, was not justified in grouping the glue-like systems, and organic colloidal jellies in general, with colloidal suspensions of metals, thus grouping colloidal gold with glue, egg-white and agar. These two types of systems have little in common. Duclaux (18), in the introduction to his book, "Les Colloïdes", states that he will not consider the metallic suspensions, as they have nothing to do with true colloids. Thomas Graham, in naming colloidal systems, had the glue-like sub-

stances in mind, for that is what colloid means. So drastic a point of view as that of Duclaux can not be taken, for it is impossible to exclude the lyophobic colloidal suspensions from the colloidal world, if only because of their historical background. Suspensions have little in common with glue-like colloids. To place the latter in the same class with the former is to fail to understand the true characters of both groups. Attempts to apply the term "emulsoid" have been the cause of much confusion. Seifriz (68) has brought this bewilderment to light and suggested, as have many chemists, elimination of the term. The concept is already dead, except in some few minds.

With visible structural features obviously too coarse to satisfy a stereochemical point of view, there remains only the molecular line of attack, that pursued by the chemists.

Biologists in their interpretation of protoplasmic structure have done no more than apply to living matter accepted theories on the structure of organic matter, of proteins, cellulose, rubber and wool. There has been some strong antagonism to all attempts to correlate the known structure of organic non-living matter and the possible structure of living matter. This opposition is apparently based on prejudice against any interpretation of the basic molecular structure of protoplasm. Investigations and hypotheses on protoplasmic structure are termed futile, just as were the experiments of Pasteur and Langley, and much other pioneer work in science. The opinion that studies on and an interpretation of the molecular structure of protoplasm are futile is held by certain chemists who are having difficulty with the structure of proteins, enzymes and pigments. It is their belief that any attempt to interpret protoplasmic structure must wait until work on non-living systems is completed. To this advice there are three replies: their work will never be done and protoplasmologists are not willing to wait that long; no branch of science has ever waited for another to advance; it has often been the less well grounded sciences which have shown the more exact sciences the road to follow, as in the cases of osmosis, ionization, solution laws, pH, hormones, vitamins and viruses. In each of these subjects the biologist set the problem and went quite a way with it, and at that point the chemist took it up. In the same way, the biologist will continue his studies on the molecular structure of protoplasm, grateful for the help of many able physicists and chemists and not much minding the destructive comments of a few.

Certain types of research have contributed much to our knowledge of protoplasmic structure, and it will be well to consider them under their respective headings.

X-Ray Analysis. Of late, X-ray analysis has contributed more to the study of protoplasmic structure than any other method of physical-chemical research; and it may also be said that one man has been primarily responsible for this research. Astbury (2, 3) has maintained a deep and constant interest in the application of his work to biology and has coordinated much of the research of physical-chemists and biologists.

The X-ray work of Astbury which has biological applications was originally done on wool, and later on a number of kinds of organic material, in particular, the muscle protein myosin. To properly understand the theories of Astbury and of others (4, 5, 37, 41) who have done similar work, it is necessary to recall both the familiar polypeptide chain and the newer concept of the cellulose fiber. Although the protein molecule is the structural unit of protoplasm, it is the cellulose fiber which first made biologists aware of the basic structure of living matter. The polypeptide chain is the older concept, but the imprint it left on structural chemistry was insufficient to carry us through to the stage now reached. The cellulose chemists succeeded in establishing the linear unit as the basic one in organic matter.

The botanist Sponsler (77) did much to bring about a revival of stereochemical thinking in biology. It was he (76) who first established with fair certainty the existence of the cellulose molecular fiber. Out of his work and that of others simultaneously carried on in Europe (37, 38, 41, 42, 78), there arose such concepts as the molecular brush heap, then a more orderly parallel alignment of fibers, and finally the overlapping of cellulose molecular threads. The last of these concepts best explains the high tensile strength of some natural fibers. The at one time popular micellar hypothesis of Naegeli has been greatly modified, and by some discarded. There is, however, considerable optical evidence based on observations made with nicol prisms, that a micellar structure is present in natural cellulose. Furthermore, the crystallites described by Astbury and others in muscle are a substantiation of the micellar hypothesis in protein.

Not only did hypotheses of the molecular structure of cellulose

lead ultimately to the concept of a fibrous structure of living matter, but microscopic observations as well made their contributions. These observations revealed minute fibrillae in natural wood. Evidence of fibrillae in natural cellulose is of various sorts. By microdissection Seifriz and Hock (73) demonstrated the presence of minute fibrils in paper pulp.

The protein fiber concept is not new, but shape and orientation of the fiber had yet to be established. This the X-ray chemists accomplished. It has long been customary to draw the diagram of a polypeptide chain in a broken zig-zag line, probably in the belief that the molecular thread is not fully extended, and this is what Astbury found to be true. But the folding is not always pronounced. The extent of folding is determined by environmental conditions. Astbury (3, 4) states that keratin, as it exists in unstretched mammalian hair, is in what he calls the α state. On stretching, the hair molecular backbones become completely extended and the keratin is then in the β state. If relaxed hair is treated with steam, the keratin threads become supercontracted. These states may not be produced by such simple foldings of the backbone as Astbury originally postulated, but the state of affairs is probably very much of this sort. Astbury (3) has presented evidence to show that myosin in relaxed muscle is in the α state, and that on muscular contraction it becomes supercontracted.

Before turning to the orientation of protein fibers in mass, which involves the important question of the lateral bond, it will be well to add evidence of another kind in support of the general concept of the molecular fiber. Included in this evidence is the excellent work of Bensley (7–10) and his collaborators (25). Their research is a chemical attack on protoplasmic structure, as distinct from the physical one of X-ray analysis. Bensley (7) sought to obtain from the cell what others had avoided because of the difficulties of solubility. Using frozen dried material Bensley and Hoerr (11) were able to introduce solvent solutions into a chemically unaltered cell. In such preparations of the liver of the guinea pig sodium chloride quickly dissolved the mobile protein of the cell but left behind a morphologically complete cell. Treatment with ammonia solution removed the mitochondria and nuclear chromatin but left the cell membrane, cytoplasmic substrate, nuclear membrane and linin threads. The cytoplasmic residue was termed "structural protein".

On repeated solution and precipitation with strong alkali a material named "ellipsin" was obtained. Later Bensley (8) reported the discovery of a sodium chloride soluble component of ellipsin, a highly viscous substance with pronounced elastic properties containing discrete fibers of great length. This he called "plasmosin", regarding it as the structural foundation of tissue very much as in myosin in muscle.

Banga and Szent-Györgyi (6) obtained a similar viscous extract from kidney which precipitated in fibrous form and gave an X-ray diagram like that of myosin β.

Purification of plasmosin by Lazarow (31) led to the discovery that plasmosin is a nucleoprotein, a fact which would suggest its origin from the nucleus. The idea that nucleoproteins are confined to the nucleus has, however, been breaking down, owing to the discovery that many active components of cells have a nucleotide structure and the discovery of Claude (15) that the particulates contain nucleoprotein. Without rejecting the idea that the nuclei contain plasmosin, Bensley (10) believes that it is, in large measure, a constituent of cytoplasm. Whether it originates there or is produced in the nucleus is, of course, a topic for speculation and research.

In contradiction to the foregoing conclusion as to the seat of origin of plasmosin, Mirsky and Pollister (44) insist that the preponderance of evidence indicates that the great bulk of fibrous nucleoprotein comes from the nucleus, that the cytoplasm can at most contain only traces of it. Furthermore, it is also maintained that this fibrous precipitate extracted by Bensley from cells only superficially resembles the well known fibrous cytoplasmic protein myosin, but conforms very closely to substances known to come from cell nuclei.

The work of Bensley and his collaborators leads to the same conclusion as does the work of the X-ray chemists, namely, a fibrous structure of the basic constituents of protoplasm. Following Bensley's suggestion (9) to modify Meyer's (42) summary of Seifriz's (69) views, we arrive at the generally accepted conclusion that certain of the structural units of protoplasm are linear molecules, or possibly aggregates of molecules, so arranged as to form a network of primary valence chains tied together at numerous points by chemical bridges held by molecular cohesion, residual valences or hydrogen bonds.

Into the biological-chemical interpretation of protein behavior has been introduced the term "denaturation". As denaturation is little understood, a general characterization of it may be given to advantage, and this is well done by Lawrence *et al.* (29) in the following words: "The conception of protein denaturation is essentially the transformation of compact, rigid almost crystalline, coiled or folded polypeptide-chain molecules with a highly specific configuration, maintained by secondary valences, hydrogen bonds, sulphydryl linkages, etc., into unrolled, more flexible, fibrillar molecules with a less specific configuration. . . . The decrease in solubility on denaturation is explained by the assumption that in the native molecule the polar groups are mostly on the external surface of the framework, but when the molecule unrolls, the non-polar hydrophobe groups are bared. The appearance of – SH groups is explained by their being no longer employed in cross-chain linkages. The crystallization of native proteins and the incapacity of denatured proteins to crystallize is explained by the view that they are no longer rigid and compact structures. On the other hand, the denatured fibrils may participate in forming regular submicroscopic bundles, or liquid crystals. . . . Drying withdraws water molecules from the framework causing it under some conditions to crumble; heat and radiant energy shatter the secondary valency bonds; shaking and gas bubbling uncoil the frameworks at the air-water interfaces; urea and guanidine derivative molecules penetrate into the frameworks and disrupt them; organic solvent molecules also penetrate and by dissolving the non-polar groups, turn the frameworks inside out".

We have, therefore, to do with two forms of the protein molecular chain, an extended and a folded form, and possibly a closed ring or globule.

We may now turn to the orientation of these chain molecules in mass. There are many possibilities: a haphazard brush heap of short fibers, an entanglement of long threads, a symmetrical orientation resembling bricks in a wall, a parallel alignment of long and overlapping rods, or a grid structure. All have been postulated, and each meets certain conditions (67). Astbury (4) advocated the grid, by which is meant that the fibers are in parallel alignment, and the plates thus formed are stacked one upon the other. In changing from the extended to the folded form, the entire grid folds

with the individual fibers. This is the basis of modern theories of muscular contraction to which reference has been made and of which more will be said later.

Into this rather well substantiated view of protoplasmic structure, there have crept some novel features, certain ones of which are enlightening, others disturbing, though not seriously so. Among the latter is the concept of the spherical molecule, so ardently supported by Wrinch (82). To build a structure possessing elasticity and related qualities out of spherical units is impossible. Comparison may again be made to a sand pile, on the one hand, and to a brush heap, on the other hand; the latter, not the former, is elastic. Meyer and Hohenemser (43) have shown that certain protein molecules, for which a spherical form has been claimed, cannot be so formed. Lecomte du Noüy (32) likewise believes that oriented albumin, the existence of which has been demonstrated by him, Devaux and Langmuir, contradicts the spherical shape. Lecomte du Noüy regards the albumin molecule as short and prismatic in shape. Banga and Szent-Györgyi (6) tentatively settled the controversy by assuming that "whenever nature needs a mobile protein (serum albumin and globulin, secretions like milk-proteins, hormones like insulin, different enzymes, etc.) it applies the globular shape, and whenever it wants to build a solid structure it applies the rod shape. Proteins have been found to be mostly globular because unconsciously the mobile and more easily accessible proteins have been selected for study".

To the convincing hypothesis of Banga and Szent-Györgyi may be added the further possibility of the conversion of the globular form into the linear form. Lawrence, Needham *et al.* (29) support this concept in saying: "If denaturation be taken as synonymous with the unrolling of a corpuscular protein, this must occur at all oil-water interfaces in the cell, e.g., at the surfaces of mitochondria, the cell membrane, the intracellular oil droplets, etc.". To this suggestion they add: "If, then, we may adopt the hypothesis that protein molecules unrolled into elongated fibrils by denaturation have a part to play in the dynamic architecture of the cell, as well as those which are elongated before denaturation, the question arises as to how they are produced in the living organism? Is it possible that the rotational churning and streaming motion of protoplasm has some connection?".

The Lateral Bond. One of the most interesting and important features of the specific type of structure here postulated, a structure the chief characteristic of which is its continuity, is the lateral bond. Seifriz (71) lays emphasis on the hydrogen bond as the most probable of lateral ties in living and non-living organic matter. He says: "The problem of the simultaneous presence in organic systems of fluidity and structural continuity is one which confronts the chemist as much as the biologist. Highly branched, cross-linked, three-dimensional polymers always exhibit elasticity, though fluid. Side chains which make lateral connections between molecules have long been recognized in stereochemistry. But the concept that one end of the link is loosely attached and the other firmly so is new and of great biological importance. It has been suggested that variations in the degree of hydration of the side chains constitute a possible explanation of a weak attachment at one end. But it is another interpretation of this feeble union which I have selected, namely, the hydrogen bond".

In a chemical cycle, as in muscle, during which accessory molecules go in and out of combination with the side chains of the protein main chains, the latter take up a cycle of configurations. In all such dynamic systems, of which protoplasm is one, there is a constant shifting of ties between the structural units. The loose union which permits this is very probably a hydrogen bond. Linus Pauling (56) believes this bond to be of greater significance for physiology than any other single structural feature. It serves our present purpose well because it is not a strong bond.

When an atom of hydrogen is attached to not one but two other atoms and thus acts as a tie between them, the union involves the hydrogen bond. In such a case, one of the points of attachment is a firm covalent bond, and the other a weaker one, essentially ionic in character. The latter is the hydrogen bond.

In general, the hydrogen bond is part of a situation in which hydrogen appears to have two valences, one stronger with some ionic character, the other weaker with more ionic character. The strength of the latter weaker bond ranges from one-tenth to one-hundredth that of the former, according to the atoms involved and their distance apart. Because it is feebly attached, the hydrogen bond shifts easily from one atom to another.

The use to which the hydrogen bond may be put in interpreting

protoplasmic behavior in respect to structural continuity is illustrated in a simple system like water. For some time it has been known that the water molecule is polar, *i.e.,* it has an electric moment. It possesses, therefore, the characteristics necessary for molecular orientation. The two hydrogen atoms are positively charged and the oxygen atom is negatively charged. At a distance, such a molecule is electrically neutral, but viewed nearby, it is positive or negative depending on one's position. The negative oxygen atom of a water molecule will attract and hold the two positive hydrogen atoms of another water molecule and with a force which may equal the hold on its own two hydrogen atoms. The valence bonds within a water molecule may be regarded as chemical and those between adjoining molecules as physical, but it amounts to essentially the same thing in the end, for the hydrogen atoms and the oxygen atoms exert forces on the surrounding molecules which are no less chemical in nature than those holding the atoms within the molecule together. Fluidity depends upon the shift that takes place between one molecule and another. Water molecules are always in partnership, but always changing partners. We thus see how polarity leads to orientation and it in turn to continuity in structure in liquid systems. That this is true is indicated by the different patterns which certain solutions yield, indicative of some kind of arrangement.

In a similar manner, Pauling interprets the structural continuity of water in terms of the hydrogen bond. Instead of the classical H_2O, it is presumed that each oxygen in water is surrounded by four hydrogens, two of which, close to the oxygen atoms, are joined to it by primary valences, and two, farther away, by the hydrogen bond.

The situation existing in water is even better applied to proteins where structural possibilities are infinitely greater; and proteins are the building material of protoplasm. In amino acids the nitrogen atom has one open coordinate position with which it may unite to a hydrogen in the same or another molecule. This union is a hydrogen bond; it may join the nitrogens of two amino acids.

The essential feature of the hydrogen bond, insofar as our present problem is solved, is its ease in shifting. This permits fluidity though maintaining continuity in structure. But protoplasm does not flow with equal ease at all times; it is often of high viscosity,

and it may be firm. The lateral bonds are, therefore, more securely held at one time than at another, and at still other times they are tighly locked, preventing flow. If the hydrogen bond satisfies these conditions, it must show considerable variability in firmness, from a feeble contact permitting ready readjustment, to a firm grip tightly locking the fibrous units of the living three-dimensional lattice. Pauling (56) points out that firmness of the hydrogen bond varies with the electronegativity of the atom.

As one of the essential points of this discussion is the variability in firmness of the weaker end of cross-chains, we may dwell upon it just a moment longer in order to emphasize an important difference between this variability in non-living and in living systems. Usually, variability in the viscosity, elasticity and tensile strength of a solution of non-living chain polymers is accomplished simply by altering the concentration. At high concentration the chains join at more points. But in protoplasm the "concentration" remains the same, yet the physical properties vary. This is accomplished by a change not in concentration but in the firmness of the cross ties. How this change may come about has been suggested. There is the further possibility that protons are added or subtracted with change in pH, forming or breaking hydrogen bonds; change in salt concentration might also alter their strengths.

The hydrogen bond provides a mechanism by means of which continuity in structure, elasticity and rapid changes in viscosity in protoplasm may be interpreted.

Long molecular chains and suitable lateral bonds make possible one of the most characteristic properties of protoplasm, its capacity to exist as a gel. A striking comparison between protoplasm and many of the more extended proteins is the capacity of both to form gels at low concentrations. Staudinger (78) has emphasized that it is not enough for a particle to be rod-shaped; to form a gel it must be able to form cross-linkages and a net structure. Chains of polystyrene are dispersed by organic solvents. Staudinger found that if as little as 0.002% of divinyl-benzene is added to the styrene before polymerization, cross-linkages are produced. The resultant polymer, although indistinguishable by chemical tests from pure polystyrene, when placed in the same solvents swells enormously but does not dissolve. In the case of a protein, like gelatin, gel formation depends on cross-linkages between the main chains. These bonds

may vary in strength, from primary valence bonds, such as salt and disulfide linkages, to van der Waals' forces.

The need of a continuous and open network in protoplasm is shown by many of its properties, among which is its high water content. Over 90% of some forms of living matter is water. If so much water is to be held by so little solid matter, the sponge-like framework must be very open and very secure.

The physical properties of living matter have been briefly reviewed by Norris (51), and somewhat more extensively by Taylor (80).

BIOLOGICAL APPLICATIONS

Anesthesia. Seifriz (70) has reinterpreted and given experimental support to the theory of anesthesia advanced by Claude Bernard. Bernard had said that anesthesia results because of the coagulation of protoplasm, but as coagulation is usually irreversible and therefore incompatible with life, the theory was discarded. The difficulty lay primarily in the use of the word coagulation. Had Bernard said gelatinization or a pronounced increase in viscosity, the theory would have attained greater standing. The lipoid solubility hypothesis replaced that of Bernard. The theory of Seifriz rests solely on experimental observations. The mechanism of anesthesia, as set forth by Seifriz (72), is one of thixotrophic setting or rapid gelatinization of the protoplasm followed by natural solation on recovery. Seifriz thus broadened the usual concept of thixotrophy by laying added emphasis on one of its features. Thixotrophy, as originally described by Schalek and Szegvary, referred to the sudden collapse of a gel due to mechanical agitation. As instantaneous resetting of the sol is equally characteristic of thixotrophic substances, thixotrophy may be defined as the instantaneous collapse and rapid regelatinization of a colloidal substance. Rapid solation and gelation both occur in protoplasm.

If the protoplasm of a myxomycete is treated with a favorable anesthetic agent, *i.e.,* one causing no observable damage and permitting recovery, such as carbon dioxide, cyclopropane and chloroform, the protoplasm ceases flow very rapidly. There is little warning, occasionally a momentary tremor, and then a sudden and complete stopping of flow. On removal of the anesthetic agent there is resumption of normal activity with no observable damage (70). The instantaneous cessation of protoplasmic streaming caused by

carbon dioxide appeared to involve a setting or gelatinization of the protoplasm. It also seemed likely that the failure of other anesthetic agents to put a sudden stop to protoplasmic streaming was an indication that no gelation had taken place, but there was no direct proof of either deduction.

By means of an ingenious technique involving the application of pressure, Kamiya (26) was able to oppose and control protoplasmic flow. If protoplasm is quiet, due to anesthesia, pressure externally applied should cause it to move if it is in a liquid state, and fail to move it if it is firm. External pressure failed to move protoplasm anesthetized with carbon dioxide.

From this we may conclude that anesthesia is due to the thixotrophic setting of protoplasm. In the case of higher vertebrates only nerves would be affected, whereas in slime molds all the protoplasm sets thixotrophically when anesthetized.

The chief virtue of a purely physical interpretation of anesthesia lies in the fact that it fits all forms of anesthesia by whatever means produced. This no chemical hypothesis can do. For example, lipoid solubility well explains the anesthetic and destructive effects of fat solvents on protoplasm, but it can not meet a situation in which a state of anesthesia is brought on by a salt, by carbonic acid or by shock. An hypothesis which states that the protoplasm of nervous tissue in higher organisms becomes firm or of very high consistency when in a state of anesthesia, no matter how it is produced, will fit all cases of anesthesia whether brought on by ether, magnesium chloride, carbon dioxide, cold or shock.

That a state of anesthesia should exist when protoplasm has been gelatinized is evident from the fact that with an increase in viscosity there must be a decrease in metabolic activity which reaches a minimum at maximum viscosity or reversible gelatinization of the protoplasm. Lhérisson (35) has pointed out that metabolic rate is inversely proportional to viscosity. He based this conclusion on the fact that the rate of chemical reactions is dependent upon diffusion and the rate of diffusion is proportional to viscosity.

An interesting field of speculation is that involving phenomena which border on surgical anesthesia as ordinarily practiced. Unconsciousness when produced by shock, cold, fear, indeed by any means, is in each case a form of anesthesia; the extremes would then be natural sleep and the coma that precedes death. The last may be

brought on by so simple a process as a sharp drop in blood acidity. Among these exceptional forms of anesthesia that by cold is recognized by the physician. Seifriz and Epstein (74) studied shock anesthesia by allowing drops of water to fall from different heights striking slime mold protoplasm. From the values for gravity, the drop mass, the height from which the drop fell, provided it did by its impact stop protoplasmic flow with subsequent recovery, were computed the force necessary to cause anesthesia by mechanical shock. Electrical shock produced similar results.

In all forms of gelation or gelatinization, and subsequent solation of protoplasm, a locking of lateral bonds, holding fibrous structural units more or less firmly together, is involved.

Genetics. The possible genetical applications of theories on protoplasmic structure are great; at least, this would seem to be inevitably true in the face of modern views on a permanent linear arrangement of genes. The ideas of continuity in protoplasmic structure, advanced by Seifriz (66), are in keeping with genetical concepts of a fixed arrangement of genes. There is less need of a mobile structure in chromosomes than in the case of cytoplasm.

As chromosomes are a form of protoplasm, they must resemble it in a general way, that is to say, the basic physical qualities of the two must be essentially the same. This is true with but minor modifications. Thus the substance of which chromosomes consists is more viscous than the surrounding medium; the viscosity apparently remains high. Chromosome material is likewise elastic and glutinous.

Buck (13) has subjected the chromosomes of the salivary gland of *Chironomus* to microdissection. He states that they show considerable rigidity, withstand a several-fold elongation without breaking, and decrease in diameter when stretched.

The visible structure of protoplasm is often alveolar (66). Metz (40) has shown that this is true of chromosomes. The giant salivary gland chromosomes of Diptera are alveolar in structure.

Permeability. The selective permeability of cells is generally handled as a subject apart from structure, but several of the classical theories of cellular permeability involve mechanisms, and mechanisms involve structure. Thus the sieve hypothesis of selective permeability is a purely mechanical interpretation; indeed chemical hypotheses such as those involving the solution of fats are, in the

last analysis, structural interpretations. The making of models which emulate the behavior of the cell membrane is likewise a problem in the physical structure of protoplasm.

It is not the reviewer's intention to summarize the subject of cellular permeability, but merely refer to several articles which help toward a better understanding of the structure of the outer surface of the cell. One of these publications is that of Lundegardh (36). He denies that materials enter cells only as molecules. In view of the modern concept of the complete ionization of salts, even at rather high concentrations which are never reached in the soil, it is doubted that there are any molecules present of the strong electrolytes needed by the plant; therefore salts must enter as ions. To cite that carbon dioxide is a weak electrolyte and but partially dissociated, has no bearing on the problem as a whole, for carbon dioxide is but one of many substances which enter cells. Lundegardh says that if a cell is placed in contact with the solution of a single salt, full equivalency in the absorption of cations and anions is an exception. "These experiments show that the salt is not taken up as molecules . . . ".

Lundegardh (36) states that the apparent contradiction of a lipoid surface film on cells and the entrance of water and ions through it, is best explained in terms of a monomolecular layer of fatty acids such as those studied by Langmuir. Rideal (60) states that such a monolayer will allow water molecules to pass through it, yet the electrical resistance must be high. In short, the conflicting experimental data are to be explained only in terms of structural characteristics of the cell surface.

Protoplasmic Streaming. An interpretation of the mechanism responsible for protoplasmic flow must certainly rest on structural features: thus, the now classical solution-gelation hypothesis of amoeboid movement, advanced by Mast (39), is based on gross structural features such as those outlined by Scarth (62) and reviewed in this paper. The rhythmic contractility hypothesis of protoplasmic streaming, advanced by Seifriz (67), is based on molecular structural features involving the contraction of folded protein chains.

Pease (57), as a result of his studies on the effects of hydrostatic pressure, suggests that protoplasmic streaming is due to local rhythmical contractions of the plasmogel.

The subject of protoplasmic streaming has been recently reviewed in this journal (Vol. 9: 49. 1943).

Frost Resistance. Scarth (63) and his co-workers (33) have given us a protoplasmic interpretation of frost resistance. They state that the key factors in resistance are protoplasmic. Other factors reinforce the protoplasmic one, but by themselves are ineffective. Most important of all is the reduced tendency of the ectoplasm to coagulate. This property protects against injury; thus it determines the minimum temperature the plant can withstand. There is no evidence that coagulation is prevented to any degree by an increase in substances such as sugars, or a decrease in pH or electrolyte concentration. The change would seem to be in the labile colloids themselves. Increased toleration of plasmolysis and increased survival after rapid deplasmolysis following non-lethal plasmolysis, invariably accompanies hardening against cold. Resistance to plasmolysis and deplasmolysis is greatest in very hardy cells and is roughly parallel to frost and drought resistance in wheat. Osmotic pressure and hardiness frequently vary independent of each other and never show the excellent correlation of protoplasmic changes.

Seifriz (72) concludes: "It is truly an encouraging sign in the progress of science when properties of protoplasm such as contractility and structural organization, which heretofore were so little understood, can now be interpreted in terms of folded polypeptide fibers, interlocking side chains, hydrogen bonds, and asymmetry of the carbon atom". To these biological processes capable of interpretation in terms of structure, may now be added anesthesia, protoplasmic streaming and frost resistance.

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THE GENETICS OF BRYOPHYTES. II[1]

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A paper under this title (7) reviewed the literature in the field approximately to 1934. The present account attempts to cover, so far as the conditions of the time render it possible, that which has since appeared.

HETEROPLOIDY

Various reports indicate the occurrence of polyploid series of related species among bryophytes as among plants of other groups. There are occasional indications also of the occurrence of different chromosome numbers in strains referred to a single species. *Riccia fluitans* has been recorded as having 8 (95) and 14 or 15 chromosomes (29). Both *Calypogeia Neesiana* and *Haplozia lanceolata* are credited with 9 (95) and 16 to 18 (29). *Dumortiera hirsuta,* previously reported with 9 (29, 45) or 10 (54), is found (89, 93–95) to occur in Japan in 3 forms having, respectively, 9, 18 and 27. The triplont is more widespread than the haplont or the diplont and appears upon a wider range of geological formations. A diploid form with 18 chromosomes is reported (89) from Tennessee. *Polytrichum commune* has 7 (35); var. *uliginosum* 7 and an unnamed variety 14 (43). Races of *Bryum caespiticium* occur with 20 instead of the typical 10 chromosomes (98, 99).

Aneuploid races are apparently infrequent in nature. In *Pallavicinia Lyellii,* whose usual haploid number is 8, 9 chromosomes were found (104, 105) in gametophytes from North Carolina and Texas. Sporophytes from the latter source had 18. In 16-chromosome sporophytes from Virginia, irregular meioses were observed. Such divisions may account for the origin of spores with unusual chromosome numbers.

Two gametophytes of *Calobryum rotundifolium* had 10 chromosomes instead of the usual 9 (78, 95). A gametophyte of *Sphaerocarpos Donnellii* with a constant chromosome number of 9 instead of 8 developed from an irradiated spore (39). Individuals of this species which are euploid in terms of autosomes but aneuploid with respect to sex chromosomes will be considered later.

[1] Supplement to article in The Botanical Review 1: 269–291. 1935.

Doubling of the chromosome number may occur in either generation. Diploid branches have appeared on haploid gametophytes of *S. Donnellii* (39). A suggestion as to the possible method of such doubling is supplied by Mackay's (52) observation of a cell in this species whose chromosomes in the diploid number were arranged in pairs. The figure is similar to some in the root tips of various angiosperms which are characterized by a spontaneous doubling of the chromosome number (polysomaty). The development of some diploid and some haploid gametophytes from the spores of one sporophyte of *S. Donnellii* led Knapp (39) to conclude that a doubling of the chromosome complement had occurred locally in the sporophyte.

Giant spore tetrads appeared in some crosses involving mutants and tropical species of *Marchantia* (15). These also were explained by a local occurrence of tetraploidy in the sporophyte. Occasional spores of *Aspiromitus sampalocensis* produced diploid gametophytes (56). These were smaller than haplonts but had larger cells, and the ♂♂ bore larger antheridia. A gametophyte of *Calypogeia Neesiana* (95) bore 1 diploid among many haploid gemmae.

By treatment of gemmae of *Marchantia polymorpha* with colchicine, Satina (cited by Blakeslee, 13) obtained diploid ♀ and ♂ gametophytes. In gametophytes of *Pallavicinia Lyellii,* after immersion in colchicine solutions, Wolcott (105) observed diploid and tetraploid cells as well as others with the original haploid complement.

Regeneration of a phenotypically ♀ diploid gametophyte from the foot of a sporophyte occurred a few times in Burgeff's (15) experiments with *Marchantia polymorpha.* He obtained similar results readily in some tropical forms of *Marchantia.* Diploid gametophytes thus produced from hybrid sporophytes (*M. emarginata* var. *multiradia* × *M. calcarea*) were mated with haploid ♂♂ of both parent forms. From the resultant sporophytes gametophytes were regenerated; all were triploid except 1 tetraplont. Spores of 1 sporophyte from a mating of this type yielded 1 triploid gametophyte (with 27 chromosomes), 1 hypotriploid (with 24), 9 diploid (with 18), 2 hyperhaploid (with 10), 2 haploid (with 9). Diploid gametophytes of *Anthoceros tjipanasanus* have likewise been obtained by regeneration (56). They were comparable to those previously reported (61) in *A. laevis* and *A. Husnoti.*

Diploid protonemata regenerated from sporophytes of *Splachnum rubrum* and *S. vasculosum* bore diploid gametophores (10). Similarly regenerated diploid protonemata of *Phascum cuspidatum* (72) produced variously abnormal shoots; these bore no archegonia, but some bore antheridium-like organs. The leaves and stems of such shoots gave rise to sporophytes from whose spores arose protonemata and gametophores. Some of the latter resembled typical gametophores; others were abnormal. It was concluded that meiosis, often irregular, had occurred in the apogamously developed sporophytes.

In polyploid series derived, respectively, from *Funaria hygrometrica* and *Physcomitrium piriforme,* the ratio of total plastid area to cell volume diminishes rapidly with increase in number of genoms (60). However, in polyploid series involving genoms from both species the diminution is much less rapid.

The most frequent cause of the appearance of diploid gametophytes, at least in hepatics, is the production by sporophytes of diploid spores. In species and races of *Sphaerocarpos* whose spores regularly remain attached in tetrads, it is well known (7) that spore dyads occasionally, but in varying proportions in different strains, replace tetrads. Less frequently triads and monads occur. Other irregular spore complexes are sometimes found (37, 39). Each spore of a dyad, or the large spore of a triad, if it germinates, usually gives rise to a diploid gametophyte. X-irradiation of spore mother cells of *S. Donnellii* before meiosis (37) results in the appearance of an increased number, approximately proportional to the radiation-dosage, of aberrant complexes.

In *Pallavicinia Lyellii,* especially in some races, occasional spore dyads occur (105). Apparently the spores of such dyads do not separate in the course of development as do the spores of tetrads in this species. Immersion in colchicine solutions of gametophytes bearing young sporophytes induced irregularities, including production of dyads and monads.

Spore dyads occur in *Marchantia polymorpha, M. alpestris* and some tropical species of the genus (15). Germination of dyad spores gives rise to diploid (rarely to haploid) gametophytes. (Apparently Burgeff's statements on this point, as well as those concerning tetrad spores, are based upon their germination before full maturity and hence before their separation.)

That the production of less (or more) than 4 spores from a spore mother cell must be consequent upon some meiotic irregularities is obvious. Nothing is known as to the nature of these irregularities in *Sphaerocarpos*. Lorbeer (45, 48) thinks that dyad-formation is due to "apomeiosis", defined as "a mitosis with the omission of a numerical reduction division". He has reported (48) artificial induction of apomeiosis in *S. terrestris* (*S. Michelii*), but without mention of the methods employed. From the sexual conditions in some of the gametophytes coming from dyad spores of *S. Donnellii*, Knapp (39) concludes that "apomeiosis" can not explain their formation. Burgeff (15) thinks that the origin of dyads in *Marchantia* is due to abnormal wall-formation after meiosis.

Only in *Calobryum rotundifolium* have the divisions in spore mother cells, leading to the formation of diploid nuclei, actually been observed (78, 95). The first division is not completed; a restitution nucleus is formed, which then divides equationally. Tatuno suggests that the checking of the first division may have been due to low temperatures at the time of its occurrence (early February).

Triploid and tetraploid sporophytes of *Sphaerocarpos Donnellii* have been obtained, the former by matings of diplont × haplont (5, 6, 39, 46, 52, 53), the latter by matings between diplonts (39, 52). In matings of (supposed) diploid with haploid gametophytes of *Marchantia* species (15), triploid as well as some diploid sporophytes were obtained. Matings of diplont × diplont produced tetraploid, triploid and diploid sporophytes. The explanation suggested for the occurrence of triplonts and diplonts is a loss of one or two genoms. Some triploid sporophytes produced tetrads of diploid spores, supposedly by addition in the sporophyte of one genom.

Somewhat abnormal sporophytes resulted in *Aspiromitus sampalocensis*, presumably from a fertilization of diploid eggs by haploid antherozoids (56). One such sporophyte produced spores which yielded 175 haploid and 42 diploid gametophytes.

MONOECISM AND DIOECISM

Any bryophytic species is either dioecious or (in a broad sense) monoecious; a spore transmits to the gametophyte arising from it one sexual potentiality only or the potentialities of both sexes. It

is relatively simple to determine which of these conditions holds in a hepatic, although in an apparently dioecious species only extensive culture can demonstrate whether or not dioecism is absolute. Monoecious mosses, however, present a variety of arrangements as concerns sex organs. The term applied to these different arrangements, as to some of which usage is not entirely consistent, are avoided in the present discussion. If both archegonia and antheridia are borne, however distributed, on the same leafy shoot (the "moss plant", here designated the gametophore), the species is monoecious, although external conditions may favor the appearance of the organs of but one sex. Greater difficulty arises when some gametophores of a species bear only archegonia, others only antheridia. Such species are often described indiscriminately as dioecious. However, if a single protonema can produce both ♀ and ♂ gametophores, the species is monoecious. If one protonema can give rise only to gametophores of one sex, the species is dioecious. Only by the culture of single protonemata, either primary (from spores) or secondary (by regeneration from gametophores), until gametophores appear, can the question of the genetic separation or nonseparation of sexual potentialities be settled. In this manner it has been shown (10), in confirmation of earlier results (14, 62), that *Splachnum pedunculatum* (*S. sphaericum*) is dioecious, and that so likewise are *S. melanocaulon, S. luteum, S. rubrum* and *S. vasculosum. S. ampullaceum* and *S. australe* are monoecious. In *Pogonatum subrotundum* (*P. nanum*) and *P. aloides,* Schratz (59) found, in addition to ♂ gametophores of varying size arising from protonemata on soil, dwarf ♂♂ growing on older gametophores (some certainly ♀). He concluded, admittedly on incomplete evidence, that the dwarf ♂♂ arose from secondary protonemata produced by the ♀ gametophores. If he is correct, these two species are monoecious.

In some dioecious hepatics, the ♀ plant is noticeably larger than the ♂. This has long been known in species of *Sphaerocarpos;* it has been shown to be true in *Aspiromitus sampalocensis* (56). In many Musci with separate ♀ and ♂ gametophores, whether the species is monoecious or dioecious, the same rule holds. In some species some or all ♂ gametophores are minute (dwarf ♂♂).

A single protonema of *Acaulon muticum* produces both ♀ and ♂ gametophores (101), the species being, therefore, monoecious. The

♂ shoots, while smaller than the ♀, are not so minute as to be designated dwarf ♂♂.

Classical examples of dwarf ♂♂ are those of *Leucobryum glaucum,* living on leaves and stems of larger gametophores of the same species. Larger ♂ gametophores, however, occur on soil. Some secondary protonemata of this species (102, 103) bore ♀ gametophores, some ♂. A single protonema never produced both. The species appears, therefore, to be dioecious. The first ♂ gametophore produced by a protonema is relatively large, though somewhat smaller than and different in habit from a fully developed ♀ gametophore. Later ♂♂ borne by the same protonema are progressively smaller; the final ones are dwarfs. In this, as in other dioecious mosses, except some species of *Macromitrium,* no constant visible differences have been detected between ♀- and ♂-producing spores or protonemata.

In *M. Blumei,* Fleischer (23) found dwarf ♂♂ in abundance on leaves and other parts of ♀ gametophores. Some of the dwarf ♂♂ arose from protonemata whose origin was traced to large spores that had germinated in locations where they might have lodged in falling from an opened capsule. No ♂ gametophores appeared on other substrata. He concluded that the spores of this and other species, including one of *Schlotheimia* and one of *Trismegistia,* are differentiated as to size and as to sexual potentialities, the larger spores being ♂- determining and the species therefore dioecious.

Fleischer's conclusions have been questioned, partly on the ground that his "large, ♂- producing" spores may have swollen in the course of germination. Goebel (24) reported that only the larger spores of *Macromitrium* germinate, and that they do not give rise to dwarf ♂♂. The small, nongerminable spores, he concluded, are abortive. Schratz (cited by Correns, 16) found that measurements of the diameters of spores of *M. Blumei* (as well as of some other, including monoecious, mosses) yield a curve with two weak maxima. Dening (17), studying herbarium material of *M. Blumei* distributed by Fleischer, found in addition to the epiphytic dwarf ♂♂, small lateral ♂ branches borne by ♀ gametophores, the species being, then, monoecious.

The most extensive studies of *Macromitrium* are those of Ernst-Schwarzenbach (19–22). Spore-measurements, whether of the area of an optical section, of the diameter (of spherical spores),

or of the average diameter (of elongated spores), gave consistent results. In some species, including *M. salakanum,* the measurements yield a sharply bimodal curve, the mature spores falling into 2 approximately equal classes, large and small. These species are classed as "heterosporous". In others, including *M. fasciculare,* the curve is unimodal with narrow dispersion. These are "homosporous". In *M. Blumei* the curve is indefinitely bi- to multimodal. This result is thus in general harmony with that of Schratz. Ten of the homosporous species are clearly monoecious, a single gametophore bearing both archegonia and antheridia. *M. Blumei,* on the basis of Dening's study, is considered to be "polyoecious", some gametophores bearing both kinds of sex organs and others (dwarf ♂♂) bearing only antheridia. Seven homosporous and 20 heterosporous species are classed as "dioecious" in the sense that they have separate ♀ (relatively large) and epiphytic dwarf ♂ gametophores. Strict dioecism is demonstrated only for *M. salakanum.* In this, as in some other heterosporous species, the large spores are green, the small spores yellow.

Protonemata from small spores of *M. salakanum* produced dwarf ♂♂; secondary protonemata from these likewise gave rise to dwarf ♂♂. Protonemata from large spores produced larger shoots resembling in structure ♀ gametophores. A few of these produced archegonia. Secondary protonemata from leaves of large shoots gave rise invariably to large shoots. It is concluded that in this species, and presumably in others resembling it, ♀ and ♂ potentialities are separated in spores, protonemata and gametophores.

Much discussed in this connection are species of *Buxbaumia,* particularly the widespread *B. aphylla.* The ♂ gametophore consists of a filament of cells bearing usually a single leaf and a single antheridium. The ♀ gametophore, while small, is more massive, bearing a few to 20 leaves and 1–5 archegonia. Dening (17) reports that secondary protonemata coming by regeneration from ♂ gametophores produced only ♂♂, and secondary protonemata from ♀♀ produced only ♀♀. This species, then, is dioecious.

To test the question of heterospory, in the sense of a difference in size between ♀- and ♂-determining spores, Dening measured spore diameters in *Bryum caespiticium, B. argenteum, Ceratodon purpureus, Mnium hornum* and *Buxbaumia aphylla,* all demonstrably dioecious; in *Diphyscium foliosum, Dicranum scoparium* and *Pogo-*

natum aloides (see also 59), doubtfully dioecious; and in *Funaria hygrometrica,* monoecious. The spore-measurements in all species gave a unimodal curve.

SEX CHROMOSOMES

Lists of dioecious bryophytes in which sex chromosomes had been recognized were published by Lorbeer (45) and Tinney (96). Since a considerable number of additional cases have been reported in the past decade, it seems desirable to present a list brought, as nearly as possible, to date. Table 1 lists those dioecious species in which sex chromosomes, recognized by differences in size and often in form, have been positively reported. In Table 2 are given those in which observation has disclosed no differences between corresponding chromosomes of the 2 sexes.

In all reported cases of sex chromosomes in bryophytes except some species of *Frullania* (see below), the ♀ possesses a single X chromosome, the ♂ a single Y. The X is larger than the Y except in *Lunularia cruciata* and *Oxymitra* (*Tesselina*) *pyramidata,* where the Y is said to be the larger.

The sex chromosomes are in most cases reported to be heteropycnotic (heterochromatic), in the sense that the whole chromosome or a considerable portion of it remains dense and deeply stained at stages (*e.g.,* prophases) at which some or all autosomes are in large part diffuse and lightly stained. This statement holds especially for the X chromosome. Observations (and probably facts) differ as to heteropycnosis in the Y. Some of the autosomes in various species, apart from those mentioned in the next paragraph, are described also as having heteropycnotic regions.

In many bryophytes, both hepatics and mosses, and in both dioecists and monoecists, one chromosome is smaller than any of the others, frequently so small that it is easily overlooked. This has been designated an "m chromosome" (29). In some mosses assumed to be diploid or triploid because of their relatively high chromosome numbers, Heitz (32) found either 2 or 3 m chromosomes. Less often there is an "M chromosome" (2 in ♀♀ of some *Frullania* species) which is noticeably larger than any of the others. A particular species may possess an m, an M, or both. Chromosomes of both these types are frequently heteropycnotic. In many cases a sex chromosome is an m or an M.

TABLE 1

DIOECIOUS BRYOPHYTES: SEX CHROMOSOMES REPORTED PRESENT

SPHAEROCARPALES	
Riella helicophylla (Bory & Mont.) Mont.	Lorbeer (44, 45)
Sphaerocarpos cristatus Howe	Allen (8)
S. Donnellii Aust.	Allen (1, 2), Wolfson (106), Lorbeer (44)
S. Michelii Bell. (*S. terrestris* Smith)	Lorbeer (44)
S. texanus Aust. (Includes *S. californicus* Aust., *S. europaeus* Lorbeer)	Schacke (57), Wolfson (107), Lorbeer (44)
MARCHANTIALES	
Conocephalum supradecompositum (Lindb.) Steph.	Tatuno (88, 95)
Lunularia cruciata (L.) Dum.	Lorbeer (45)
Marchantia diptera Mont.	Tatuno (84, 95)
M. "grisea"	Haupt (26)
M. polymorpha L.	Haupt (25), Lorbeer (45), Tatuno (84, 95)
Oxymitra pyramidata Hüben. (*Tesselina pyramidata* Dum.)	Lorbeer (45)
Riccia Bischoffii Hüben.	Lorbeer (45)
R. Curtisii (Lindb.) T. P. James (*Thallocarpus Curtisii* Lindb.)	Lorbeer (45), Siler (71)
JUNGERMANNIALES ANACROGYNAE	
Calobryum rotundifolium (Mitt.) Schiffn.	Tatuno (73, 74, 95)
Moerckia Blyttii (Moerck) Brockm.	Lorbeer (45)
M. Flotowiana (Nees) Schiffn.	Lorbeer (45)
Pallavicinia longispina Steph.	Tatuno (73, 74, 95)
P. Lyellii (Hook.) S. F. Gray	Tatuno (75, 79, 95)
P. radiculosa (Sande) Schiffn.	Lorbeer (45)
Pellia Fabbroniana Raddi	Heitz (30, 31), Tatuno (73, 75, 79), Lorbeer (45)
P. Neesiana (Gottsche) Limpr.	Showalter (69, 70), Heitz (32), Tatuno (73, 75, 79), Lorbeer (45)
Riccardia (*Aneura*) *blasioides* Horikawa	Tatuno (76, 79, 95)
R. Makinoana (Steph.)	Tatuno (92, 95)
R. pellioides Horikawa	Tatuno (83, 95)
R. pinguis (L.) S. F. Gray	Tatuno (76, 79, 95)
JUNGERMANNIALES ACROGYNAE	
Calypogeia suecica (Arn. & Perss.) K. Müll.	Lorbeer (45)
Frullania dilatata (L.) Dum.	Lorbeer (45), Tatuno (82, 83, 95)

TABLE 1—(*Continued*)

F. Fauriana Steph.	Tatuno (81, 95)
F. japonica Sande	Tatuno (81, 95)
F. nishiyamensis Steph.	Tatuno (88, 95)
F. sakawana Steph.	Tatuno (88)
F. squarrosa (R. Bl. & N.) Dum.	Tatuno (89, 95)
F. viridis Horikawa	Tatuno (83, 95)
F. yakushimensis Horikawa	Tatuno (83, 95)
Plagiochila asplenioides (L.) Dum.	Lorbeer (45)
ANTHOCEROTALES	
Aspiromitus sampalocensis (Burgeff)	Rink (56)
A. spec. "Singapore"	Rink (56)
BRYALES	
Ceratodon purpureus (Hedw.) Brid.	Heitz (33), Shimotomai and Kimura (63, 64), Jachimsky (35)
Mnium punctatum (L.) Hedw.	Jachimsky (35)[1]
Pogonatum grandifolium (Lindb.) Jaeger	Kurita (43)
P. inflexum Lindb.	Shimotomai and Koyama (65, 66)
P. spinulosum Mitt.	Kurita (43)
Polytrichum formosum Hedw. (*P. attenuatum* Menz.)	Shimotomai and Kimura (63, 64)

[1] Jachimsky finds a probably but not certainly unequal (XY) pair also in *Mnium hornum* L., *Bryum argenteum* L. and *B. capillare* L.

In all Sphaerocarpales thus far investigated, the Y is an m chromosome. In *Sphaerocarpos cristatus* and *Riella helicophylla,* the X, while much larger than the Y, is no larger than some of the autosomes—therefore not to be classed as an M. In all other species of *Sphaerocarpos* studied, the X is an M chromosome.

Haynes (27) concluded that *S. texanus* and *S. californicus,* both named by Austin, should be combined, the name *texanus* having priority. Lorbeer (45) has attempted to distinguish between the American and European forms referred to this species on the grounds of their incompatibility and of a difference in the structure of the X chromosome. The distinctions are based apparently on the study of a single strain from each source, and are obviously inadequate as taxonomic criteria. On the other hand, an examination of spores from various parts of the United States indicates that *texanus* is really a complex, ultimately to be divided into two or or more varieties or species. From Reimers' (55) study, it appears that similar differences occur among European races. For pres-

TABLE 2

DIOECIOUS BRYOPHYTES: RECOGNIZABLE SEX CHROMOSOMES REPORTED NOT PRESENT

MARCHANTIALES	
Conocephalum conicum (L.) Dum. (*Fegatella conica* Corda)	Showalter (67), Lorbeer (45), Tatuno (86, 95)
Marchantia cuneiloba Steph.	Tatuno (84, 95)
M. radiata Horikawa	Tatuno (84, 95)
M. tosana Steph.	Tatuno (84, 95)
Riccia Curtisii (Lindb.) T. P. James (*Thallocarpus Curtisii* Lindb.)	McAllister (51)
JUNGERMANNIALES ANACROGYNAE	
Blasia pusilla L.	Lorbeer (45)
Cavicularia densa Steph.	Tatuno (95)
Haplomitrium Hookeri (Sm.) Nees	Heitz (32), Lorbeer (45)
Makinoa crispata (Steph.) Miyake	Tatuno (86, 87, 95)
Moerckia hibernica (Hook.) Gottsche	Lorbeer (45)
Petalophyllum Ralfsii (Wils.) Gottsche	Lorbeer (45)
Riccardia pinguis (L.) S. F. Gray (*Aneura pinguis* (L.) Dum.)	Showalter (68), Lorbeer (45)
JUNGERMANNIALES ACROGYNAE	
Alicularia scalaris (Schrad.) Corda	Lorbeer (45)
Chandonanthus birmensis Steph.	Tatuno (90)
Eucalyx ovicalyx (Steph.) Horikawa	Tatuno (77, 95)
Frullania aoshimensis Horikawa	Tatuno (85, 95)
F. densiloba Steph.	Tatuno (85, 95)
F. Makinoana Steph.	Tatuno (85, 95)
F. moniliata (R. Bl. & N.) Mont.	Tatuno (82, 85, 95)
F. Tamarisci (L.) Dum.	Lorbeer (45)
Leptoscyphus verrucosus (Lindb.) K. Müll.	Tatuno (91, 95)
Madotheca (*Porella*) *densifolia* Steph.	Tatuno (87, 95)
M. japonica Sande	Tatuno (87, 95)
M. parvistipula Steph.	Tatuno (92, 95)
M. Perrottetiana Mont.	Tatuno (92, 95)
M. tosana Steph.	Tatuno (92, 95)
M. ulophylla Steph.	Tatuno (87, 95)
M. ulophylla var. *"sacculosa"* Horikawa	Tatuno (87, 95)
Odontoschisma denudatum (Mart.) Dum.	Tatuno (91, 95)
Plagiochila adiantoides (Swartz) Lindb.	Johnson (36)
Pleurozia giganteoides Horikawa	Tatuno (95)
Porella: see *Madotheca*	
Radula Lindbergiana Gottsche	Lorbeer (45)
Schiffneria viridis Steph.	Tatuno (95)
BRYALES	
Barbula unguiculata (Huds.) Hedw.	Jachimsky (35)
Bryum caespiticium L.	Jachimsky (35), Wettstein and Straub (100)
Mnium Maximowiczii Lindb.	Shimotomai and Koyama (65, 66)
[2]*Pogonatum aloides* (Hedw.) Palis.	Jachimsky (35)

TABLE 2—(*Continued*)

[2]*P. subrotundum* (Huds.) Lindb. (*P. nanum* (Schreb.) Palis.)	Jachimsky (35)
P. urnigerum (L.) Palis.	Jachimsky (35), Kurita (43)
Polytrichum commune Hedw.	Jachimsky (35)
P. juniperinum Hedw.	Jachimsky (35), Kurita (43)
P. piliferum Hedw.	Jachimsky (35)
Rhizogonium Dozyanum Lac.	Kurita (43)

[2] If Schratz (59) proves correct in considering these two species monoecious, they, and possibly also *P. urnigerum*, should not be included here.

ent purposes, all members of this complex are treated as *texanus*. All that have been studied cytologically have similar sex-chromosome complements.

In all cases of sexually differentiated chromosomes reported in Marchantiales, except in *Riccia Curtisii*, both X and Y are m chromosomes. In *R. Curtisii*, McAllister found no differentiated sex chromosomes; each sex has an m chromosome. Lorbeer and Siler find a very small Y in the ♂; the X of the ♀ is no larger than some of the autosomes. The discrepancy appears to be explained by the fact that two distinct forms which have been referred to this species occur in the southern United States (28, 58). A form apparently identical with one of these is found also in South Africa (18).

Among various races of *Marchantia polymorpha* studied by Haupt (25) are 3 forms found in nature and six "mutations" (apparently derived from "forma *typica*"). In some of these races she detects no certain difference between the m chromosomes of ♀ and ♂. Since, however, these chromosomes clearly correspond to the X and Y in races in which a difference is apparent, Haupt holds them also to be sex chromosomes. The same argument may hold for the m chromosomes of several tropical species regarding whose size differences she is noncommittal. A like suggestion has been made (45) as to the m chromosomes of *Conocephalum conicum*, and may be applied to the 3 dioecious species of *Marchantia* studied by Tatuno (Table 2) in which also no size differences could be recognized between the m chromosomes of ♀ and ♂.

In the anacrogynous Jungermanniales, except that the X is in all reported cases larger than the Y, no definite rule holds, even within

a single genus. In *Riccardia pellioides, R. blasioides* and *R. Makinoana,* the X is the M chromosome. In *R. blasioides,* the Y is the smallest (m). In the other 2 species, the Y is apparently not the smallest. In *Pallavicinia Lyellii* and *P. Flotowiana,* X is not the largest and Y is not the smallest. In *P. radiculosa* both X and Y are m chromosomes.

In *Riccardia pinguis,* Showalter and Lorbeer found no recognizable sex chromosomes. Tatuno finds an X, one of the largest chromosomes, and a Y, smaller but not the smallest in the set. Probably the Japanese plants studied under the name by Tatuno are not the *R. pinguis* of the United States and Europe.

In *Calypogeia suecica* and *Plagiochila asplenioides,* of the acrogynous Jungermanniales, both X and Y are m chromosomes. The frullanias found to possess sex chromosomes, alone among bryophytes thus far investigated, have an X element consisting of two chromosomes (X_1 and X_2) and a single Y chromosome. X_1, X_2 and Y are much larger than any of the autosomes. In *F. ornithocephala,* all plants grown from spores remained sterile (45). Some, presumably ♀, had 3 large heteropycnotic chromosomes; others, presumably ♂, had 2.

In the dioecious frullanias in which sex chromosomes are not recognizable (Table 2), there is in each sex one heteropycnotic M chromosome and 1 heteropycnotic m, the latter not present in those species with 2 X's and a Y. The species with and those without differentiated sex chromosomes belong, respectively, to distinct subgenera.

Tatuno (80, 95) suggests that, since many monoecious hepatics possess heteropycnotic M or heteropycnotic m chromosomes or both, and since the X and Y chromosomes of dioecious species (commonly heteropycnotic) are sometimes M and sometimes m chromosomes, sex chromosomes may have originated from 2 sources. In those cases in which the X or the Y is not distinguishable by size from some of the autosomes, Tatuno's hypothesis might be supported by the assumption of changes in size resulting from translocation.

In the two dioecious Anthocerotales with X and Y, both are m chromosomes.

Among mosses, conditions vary much as they do among hepatics. In the 3 species of *Pogonatum* reported to have sex chromosomes, and in *Polytrichum formosum,* the X is an M, the Y an m chromo-

some. In *Ceratodon purpureus,* both are M chromosomes. In *Mnium punctatum,* both are m chromosomes.

Lorbeer (44, 45) has figured the X and Y as more or less widely separated at early metaphase of the first meiotic division in *Sphaerocarpos Donnellii,* the autosomes being still intimately paired. He concludes that there is never a close pairing of X and Y, and hence no possibility of a cross-over between them. Their regular passage to opposite poles is unexplained. A like condition is described also (46), involving the 2 X's and the Y of a triploid sporophyte. Similar "distance conjugation" is postulated (45) for the sex chromosomes of other species of *Sphaerocarpos* and for a pair of "wholly heterochromatic" chromosomes in each of a considerable number of hepatics and mosses. In the latter cases the evidence consists in figures showing the bodies in question separated, while the members of each other pair are still in contact. Such figures, of course, may well result from an early separation of a closely conjugated pair. Distance conjugation is considered as related to the heterochromatic character of the chromosomes in question rather than to their function in sex-determination.

Heitz (34) criticizes this conception. He refers to various figures of other workers showing a close pairing of heteropycnotic sex chromosomes, as well as to certain of Lorbeer's own figures (44) which represent the X and Y as connected by a conspicuous strand.

Studies by Mrs. Duncan (unpublished) show that in *S. cristatus* there is a close pairing of the X and Y chromosomes in meiosis, "distance conjugation" here being clearly out of the question.

HETEROPLOIDY AND SEX

In *Sphaerocarpos Donnellii,* each spore of a dyad, or the large spore of a triad, commonly possesses, as shown by the gametophyte to which it gives rise, the diploid number $(2A + X + Y)$[3] present in the sporophyte. Gametophytes with this chromosome complement are in appearance and to some extent functionally ♀. However, they produce ocassional intersexual organs (6, 52), never reported in gametophytes without a Y. Lorbeer (46, 48) says that in the combination $2A + X + Y$, the Y disappears during germination of the spore, the X being later doubled and the

[3] "A" represents the haploid set of autosomes characteristic of the gametophyte. The numerical value of A varies with the species.

plant being finally ♀ with 2A + 2X. That such changes are not of general occurrence is shown by Lorbeer's (44) own study of gametophytes with both X and Y, as well as by many observations in this laboratory. Knapp (39), in both X-irradiated and non-irradiated material, obtained, from dyad spores, 2A + X + Y gametophytes whose chromosome complement persisted without change. In a different category (37, 39) were gametophytes with A + X + Y from irradiated spores which gave rise to ♀ branches with A + X and to ♂ branches with A + Y. In gametophytes from dyad spores of *S. terrestris* (*S. Michelii*), "apomeiosis" having been induced, Lorbeer (48) finds that, contrary to what he thinks occurs in *S. Donnellii,* the full complement of 2A + X + Y persists.

In both irradiated and unirradiated material of *S. Donnellii,* while dyads from sporophytes developing between November and March gave rise only to the expected 2A + X + Y gametophytes (39), those from sporophytes developing between August and October produced predominantly 1 ♀ with 2A + 2X and 1 ♂ with 2A + 2Y. Triads from these latter sporophytes yielded, when all spores germinated, either 2 ♀♀, 1 ♂ or 1 ♀, 2 ♂♂. Often of the 3 gametophytes from a triad, 2 were diploid, 1 haploid. In some instances all 3 were haploid. Apparently, seasonal conditions may influence the course of divisions in the spore mother cell. This may explain previously known occurrences of ♀♀ with 2A + 2X and ♂♂ with 2A + 2Y (7).

Matings of diploid × haploid *S. Donnellii* (5, 53) yielded triploid sporophytes (3A + 2X + Y). The spores from only one family germinated in numbers. Of the tetrads giving complete germination, some yielded 2 ♀♀, 2 ♂♂, some 4 apparent ♀♀. Seven gametophytes survived for cytological study; 6 were apparent ♀♀ with 2A + X, the possibility not being entirely excluded of an undiscovered Y in some; 1 was a ♂ with 2A + 2Y. An apparent ♀ from a tetrad spore from another similar mating had 2A + X + Y (52). The failure of many spores to germinate and the early death of many gametophytes may well have been due to aneuploidy. Another mating of diploid × haploid with similar results is reported (39).

Mating of a diploid ♀ (2A + X) with a diploid ♂ (2A + 2Y) yielded a sporophyte producing spore tetrads (52). Each of 2 ♂ gametophytes from the spores had a chromosome complement of

2A + 2Y. Two ♀♀ had 2A + X; 1 apparent ♀ (producing intersexual organs) had 2A + X + Y.

The following chromosome complements are shown to be compatible with viability in *S. Donnellii:* Gametophytic, ♀, A + X, 2A + X, 2A + 2X; intersexual, A + X + Y, 2A + X + Y; ♂, A + Y, 2A + 2Y. Sporophytic, 2A + X + Y, 3A + X + Y, 3A + X + 2Y, 3A + 2X + Y, 4A + X + 2Y, 4A + X + 3Y.

Dyad spores of *Marchantia polymorpha* and *M. alpestris* (15) yield ♀♀ (2A + 2X) and ♂♂ (2A + 2Y). Those of some tropical marchantias produce in addition some gametophytes phenotypically ♀, having presumably the equivalent of 2A + X + Y. Since in these tropical species X and Y can not certainly be recognized by differences in size (25), the small heteropycnotic chromosome in either sex is assumed to be functionally an X or a Y.

Of diploid gametophytes from spores of *Aspiromitus sampalocensis* (56), 28 produced antheridia. These ♂♂ had 2 Y chromosomes. The remaining 47 were presumed to be ♀♀, although only a few bore somewhat abnormal archegonia, usually but 1. Each of 2 producing archegonia had an X and a Y.

Regenerated diploid protonemata of *Splachnum rubrum* (10) produced only ♀ gametophores; but some of these bore paraphyses, typical of ♂♂. Of 2 diploid protonemata of *S. vasculosum,* similarly obtained, 1 produced only ♀ gametophores, of which 1 bore a paraphysis and 1 bore perichaetial leaves resembling those of a ♂. The other protonema produced many ♀ and a few ♂ gametophores.

Diploid monoecious gametophytes regenerated from the sporophytes of dioecious mosses are usually largely or wholly sterile. An instance has been described in *Bryum caespiticium* in which such sterility was overcome (98–100). The rare sporophytes borne by the regenerated gametophytes of this species produced mostly non-viable spores. The spores which germinated yielded chiefly diploid monoecious gametophytes similar to the parent plant; some gave rise to diploid ♀♀, diploid ♂♂, haploid ♀♀, haploid ♂♂, "monstrous" diploid monoecists, hypo- and hyperdiploid monoecists, and sterile plants. One monoecious diplont behaved differently from all others. In its first year it bore only antheridia, the next year and thereafter both antheridia and archegonia. Only embryonic sporophytes developed in the ear-

liest archegonia. In later years, normally developed sporophytes appeared; their number increased until in the course of 11 years the plant became fully fertile, producing viable spores all developing into diploid gametophytes of constant type. Gradually also the sizes of cells and organs diminished from those typical of *gigas* forms, finally reaching approximately the sizes characteristic of the original haploid parents. Portions separated from this diplont gave rise to clones which were similarly multiplied. Strains were grown also from spores, and these strains in turn were reproduced through spores. All clones and strains obtained in these ways were similar to the parent diplont, and all followed a parallel course of diminishing cell size and increasing fertility. The new constant, fertile diploid race is designated *B. Correnssii.*

Cytological study (100) showed that each of the 10 gametophytic chromosomes of typical *B. caespiticium* is duplicated in *B. Correnssii.* In meiosis in *B. Correnssii,* 20 chromosome pairs are present. There are no trivalent or quadrivalent groups, but evidences of secondary association of like pairs. In a wild diploid monoecious form of *B. caespiticium,* the meiotic behavior is similar. Wettstein thinks that the increase in fertility was a consequence of the diminution in cell size, this in turn being due to a dominant mutation. The exclusive formation of chromosome bivalents in meiosis in *B. Correnssii,* whereas the original diploid form derived by regeneration gave rise to sporophytes which, as indicated by the variance among their progeny, produced trivalents and quadrivalents, is explained by the occurrence in the spore giving rise to *B. Correnssii* of a genic combination which induces bivalent-formation. The appearance in nature of diploid races with cells similar in size to those of *B. Correnssii* suggests that gradual changes such as were observed in this race may occur in spontaneously produced diplonts.

INTRA- AND INTERSPECIFIC COMPATIBILITY

Full compatibility is found between ♀♀ and ♂♂ of strains of *Sphaerocarpos Donnellii* from various localities (7, 9). The same is true as between all ♀♀ and ♂♂ within a single strain, and between ♀♀ and ♂♂ from spores of a single tetrad. The productivity of matings between typical and mutant clones seems to be limited only by the relative fertility or infertility of the mutants.

Most attempted matings between plants of different species of

this genus have been unsuccessful. However, sporophytes have appeared (9) in crosses of *S. Donnellii* (♂) with 2 strains of *S. texanus* (♀). The spore tetrads produced by the hybrid sporophytes showed wall markings closely similar to those of the ♀ *texanus* parents. Most spores failed to germinate. A few from one sporophyte which germinated produced 3 mature gametophytes, 2 ♀♀, 1 ♂. These gametophytes were interfertile, and 1 ♀ and 1 ♂ yielded offspring in matings with clones of *S. Donnellii*.

A diploid ♀ (2A + 2X) *S. texanus* was crossed (39) with a haploid ♂ (A + Y) *S. Donnellii*. Among the gametophytic offspring were apparent ♀♀ which by elimination of an X chromosome produced ♂ branches, and intersexual and sterile types which changed to normal ♀♀ or ♂♂.

MUTATIONS INVOLVING SEXUAL CHARACTERS

In the previous review it was noted that (up to 1934) the problem of sex-determination in dioecious bryophytes appeared simple; by no means then discovered could a gametophyte of one sex be induced to manifest characters of the opposite sex. In species in which sex chromosomes could be identified, the presence of an X or of 2 X's implied femaleness; of a Y or of 2 Y's maleness; of an X and a Y, intersexuality or monoecism. More recent results suggest that the genetic situation may be somewhat more complicated than these statements imply.

Some cases of apparent sex-reversal already cited are explainable by the loss of an X or a Y chromosome. In the same category is a case (39) in which spore tetrads of triploid sporophytes of *Sphaerocarpos Donnellii* yielded in some cases 4 apparent ♀♀. Certain of these gametophytes produced ♂ branches. The explanation offered is that in a complement of 2A + X + Y the X chromosome was now and then eliminated.

From a mating of an apparently ♀ diplont from an interspecific *Marchantia* hybrid with a haploid ♂ of one parental species, a spore was obtained (15) which gave rise to a triploid ♂. From a mating of an apparently ♀ haplont (likewise hybrid and assumed to have the equivalent of an X and a Y) with a ♂ of a parental species, a diploid ♂ was obtained. In both cases it is suggested that either an X chromosome was lost or that it mutated to a Y. The occurrence of diploid ♀♀ derived from a mating of an apparently ♀ diplont (as-

sumed to have an X and a Y) with a ♂ diplont is explained by the mutation of a Y to an X. Male lobes appeared on diploid apparently ♀ gametophyptes derived from crosses involving several tropical species of *Marchantia*. Of such ♂ lobes, isolated, 22 developed into fully ♂ plants. Fourteen produced archegonia, 2 of them antheridia also. In addition, outgrowths combining ♀ and ♂ characters appeared on otherwise ♀ heads of *M. planiloba*. Cytological examination of plants developed from the aberrant ♂ lobes showed the full diploid number of chromosomes present. Burgeff suggests the mutation of an X to a Y chromosome. It may, however, be noted that the phenomena here are comparable to those in regularly monoecious bryophytes in which one gametophyte bears organs of both sexes variously distributed and may, as in several mosses, produce intersexual structures also.

More conclusive than these results with species of *Marchantia,* in which, incidentally, X and Y chromosomes are not certainly recognizable, are those from irradiation experiments with *Sphaerocarpos Donnellii*. Knapp's (37) X-irradiation of spore mother cells shortly before meiosis, or of young spore tetrads, resulted in the production from some tetrads of 1 ♀, 3 ♂♂ instead of the expected 2 ♀♀, 2 ♂♂. Tetrads of which only 3 spores germinated sometimes yielded 3 ♂♂. A few apparently similar results were obtained by irradiation of mature tetrads. When 3 ♂♂ arose from spores of a single tetrad, it was found that 1, sometimes distinguishable by greater size of cells and organs, possessed an X chromosome. In some instances, possibly in all, a variable portion of this chromosome had been lost. Evidently the sexual character of the spore had been reversed. In no instance was there a reversion from ♂ to ♀. Nor in any case save for a few tetrads from one sporophyte irradiated while still very young, were both of the potential ♀♀ of a tetrad changed to ♂♂. In these latter instances, Knapp suggests, radiation was effective while the X chromosome was functionally single. The ♂♂ resulting from reversion are sterile; their antherozoids are only weakly motile.

Lorbeer (47) X-rayed the growing tips of ♀♀. Later subjection of the plants to moisture and reduced illumination induced the appearance of regenerative shoots, of which a small minority were ♂. In one instance, the continued growth of the irradiated tip bore 2 longitudinal rows of antheridia and 1 row of archegonia. The

antheridia on the ♂ plants or parts so produced are somewhat larger than those on normal ♂♂. Irradiation of ♂♂ resulted in no change of sex (49); but sterile mutants were obtained by irradiation of either sex.

After irradiation of ♀♀ with A + 2X chromosomes, 3 monoecious gametophytes appeared which produced alternately ♀, ♂, and intersexual organs. In 1 of these mutants 1 X remained intact, the other was represented by a fragment. Occasionally the fragment disappeared in a cell or cells of the apical region, and the succeeding growth, containing only the intact "♂ X" (plus the autosomes), was ♂. A sporophyte resulting from a mating of this monoecious plant with a typical ♂ produced spores some of which gave rise to ♂♂ having the ♂ X and a Y. These gametophytes produced motile antherozoids, whereas the antherozoids borne by plants with a ♂ X and no Y were non-motile. The other 2 monoecious mutants were similar, except for translocations involving the ♂ X and some of the autosomes.

In *Riella helicophylla* a spontaneous mutation occurred in a haploid ♀ gametophyte (45, 47), which bore archegonia on its lower, antheridia on its upper part. The cells of the ♂ portion, including those of the antheridia, still possessed the ♀ chromosome complement (A + X). X-irradiation (49) induced a similar change of ♀ plants to the monoecious condition; in a few instances irradiation resulted in a change to complete maleness. All plants, whether ♀, monoecious, or ♂, possessed like chromosome complements. Similar experiments with ♀ *Marchantia polymorpha* yielded one monoecious and one ♂ plant, the latter with non-motile antherozoids. In these plants also the X chromosome persisted unchanged.

MUTATIONS INVOLVING VEGETATIVE CHARACTERS

Many structural mutations affecting other than sexual characters have been observed in *Sphaerocarpos Donnellii*. However, many of the mutants, whether spontaneous or induced, produce diminished numbers of sex organs, which are often largely nonfunctional. Typically, this, like other species of the genus, bears archegonia or antheridia profusely and continuously from a very early stage for as long as vegetative growth persists. *Tufted* strains (3) seem to be fully fertile. The *vegetative* race (9) bears a proportionally smaller number of sex organs and produces an

even smaller proportion of sporophytes. Vegetative ♂♂, however, seem fully interfertile with typical ♀♀. The *semisterile* character, known only in ♂♂, involves a still smaller proportion of sex organs. The *cupulate* character, also known only in ♂♂, involves a low degree of fertility. *Dwarf* ♂♂ have proved wholly infertile. *Sterile* males bear no antheridia. The *polycladous* race has fully fertile ♂♂, but the ♀♀, with only rare normal-appearing archegonia, are wholly sterile. So far as they have been studied, these mutants possess chromosome complements indistinguishable from those of the typical form of corresponding sex. This has been shown for semi-sterile and polycladous ♂♂, polycladous ♀♀ (106), dwarf and cupulate ♂♂ (52). A spontaneous sterile mutation is reported (47) in a ♀ clone.

Mutations showing structural aberrancies resulted from X-irradiation (37); their number was approximately proportional to the dosage. With equal dosage, the proportion of mutations was highest following irradiation of young spore tetrads shortly after meiosis, lowest when mature tetrads were irradiated, intermediate when radiation acted shortly before meiosis. One ♀ whose X chromosome lacked part of one arm (38) differed structurally from a typical ♀. When this was mated with a typical ♂, each tetrad of the resultant sporophyte gave rise to 2 ♀♀ aberrant like the mother and 2 typical ♂♂. The morphological mutation then is sex-linked. Of 16 mutations in ♀ gametophytes, not investigated cytologically, 9 proved similarly to be sex-linked; of 8 mutations in ♂ gametophytes, none were sex-linked. Some aberrant gametophytes (39) produced branches that approached the typical form—a change explained by the elimination of autosomes from a hyperhaploid complement.

Ten mutants (40), some spontaneous, some appearing after irradiation, were mated with typical gametophytes. As in the earlier results of the present writer (3, 4), some tetrads gave rise to but 2 genetic types of gametophytes in terms of sex and of the mutant character involved, some to 4 types. In matings involving each of 3 mutant characters, each tetrad gave rise to but 2 types; in those involving each of 7 mutant characters, differing proportions of tetrads produced 4 types each. Of 13 mutations which appeared (42) after exposure of antherozoids to ultra-violet light, 10 were lethal or sublethal.

By α- and X-irradiation of spores of *Physcomitrium piriforme* (11), over 100 mutant forms were obtained. Many of these, from the facts that only about 30% bore sex organs, and that only about half of these produced sporophytes, are assumed to be heteroploid. Those which proved fertile varied greatly in characters of protonema, gametophore, and sporophyte. The species is monoecious, antheridia being borne typically at the end of the main stem of the gametophore, archegonia at the end of a lateral branch. Some mutants bore both antheridia and archegonia in a single head.

GENETIC ANALYSIS

Knapp (40) concludes that the X chromosome of *Sphaerocarpos Donnellii* bears a gene or genes tending to femaleness. By irradiation such gene, or a sufficient proportion of such genes, may be inactivated, or may be lost with the elimination of the corresponding part of the chromosome. Since ♂♂ appear with the X chromosome thus modified and with no Y, a tendency to maleness is determined by a mechanism borne, not on the Y, but on some of the autosomes. Since the unmodified X is present only in the ♀, but a small portion of it at most being represented in the ♂ by the Y, Knapp argues that the greater part of the X lacks genes necessary to the existence of the gametophyte. This is further evidenced by the viability of occasional ♀♀ lacking various parts of the X. On the other hand, the fact that 9 of 16 radiation-induced structural mutations in ♀♀ proved to be sex-linked indicates that the genes involved in these mutations are borne on the X. This "totally heterochromatic" chromosome therefore carries genes which influence structure but are not essential to the life of the gametophyte. Genes involved in the 7 non-sex-linked mutations in ♀♀ must be borne on autosomes. Since all of 8 analyzed mutations in ♂♂ were non-sex-linked, there is no evidence that the Y chromosome carries genes affecting structure.

Linkage studies (41) established 4 groups (respectively 7, 2, 2 and 1) of genes presumably borne on 4 of the 7 autosomes. Knapp concludes that separation of the centromeres of each pair of homologous chromosomes occurs always in the first meiotic division, and that, as in other organisms studied, crossing over involves only 2 of the 4 chromatids of each chromosome pair.

Lorbeer (49, 50) postulates a ♀-tending gene (γ) on the X

chromosome of *S. Donnellii;* this is borne near the centromere, since it persists after the elimination of various parts of either arm of the chromosome. The Y chromosome bears a ♂-tending allel (a). The effects of irradiation upon sex expression show that a γ gene may be changed to σ, an intermediate allel for monoecism (*Riella, Marchantia*), or directly to an a allel (*Riella, Marchantia, Sphaerocarpos*). All sex-mutations thus far obtained are in the direction $\gamma \rightarrow \sigma \rightarrow a$. Elimination of portions of the X chromosome leads to subvitality or lethality unless the Y also is present. In the latter case, a deletion of part of the X is compensated. It follows that the Y possesses a complement of genes (except for γ) approximately equivalent to that borne by the X. The great difference in size between X and Y is explained by greater distances between genes located on the X. Determining for sex dimorphism is, in the ♂, the reaction system of the autosomes plus the a gene; in the ♀, probably the autosomal reaction system plus the γ and other genes in the X chromosome. Since ♂♂ resulting from irradiation, possessing a modified X and no Y, produce non-motile or very slightly motile antherozoids, the Y chromosome must bear a "mobilis" gene.

Reviewing previous work on polyploidy, Wettstein (99) points out that, while cell volume usually increases with the induction of auto- or allopolyploidy, the ratio of increase varies greatly in different polyploid combinations. In some allopolyploid series in mosses, cell size actually diminishes with an increase in number of genoms. It is concluded that the character of cell size in polyploid combinations is influenced by the interactions of parental genes as well as by the quantitative effect of an increase in chromosome number. A similar conclusion is reached by Barthelmess (12) on the basis of comparisons between haploid mutants of *Physcomitrium piriforme* and diploid races obtained by regeneration from the haplonts.

Discussing his earlier studies of interspecific and intergeneric moss hybrids, Wettstein (97) repeats his previously cited views (7) as to the genetic importance of the cytoplasm. Genom and plasmon, he emphasizes, are parts of the idioplasm, different in kind but equal in importance. Differences in cell size between reciprocal hybrids are ascribed to cytoplasmic influence (99).

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THE CYTOLOGY OF HOST–PARASITE RELATIONS. II[1]

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This review is a follow-up of my discussion in The Botanical Review, September 1935, upon the physiology of parasitism with special reference to rust fungi. In that discussion the haustorium was considered an index of the high degree of adaptation effected by the parasite in that the increased feeding surface which it affords is gained, usually, without lysis of the host-cell walls or penetration of the primordial utricle and with a minimum of disturbance of host-cell metabolism. This adaptation is indicated by the general lack of chlorosis in the host tissue during the vegetative stages of the rust, and often by the actual retention of chlorophyll in infected areas as seen in the "green island" phenomenon. In contrast to this trend toward mutualism in a susceptible host the lack of adjustment in a resistant host is evidenced by chlorosis, altered metabolism and death of host cells, and by various stages of sheath formation in the parasite.

In the past decade the trend of work upon rusts has seemed to be toward cultural and biochemical rather than microscopic methods. Among the few studies which carry on the older tradition, Saville has presented a detailed microscopic study of nuclear structure and behavior in species of the Uredinales. She finds two distinct types of nuclei: the unexpanded migration nucleus of the vegetative mycelium including the haustorium, and the expanded form where the chromatin becomes distributed in an ectosphere. The latter are the nuclei concerned in spore formation (28).

McKenzie reports an experimental short cycle infection with *Cronartium quercuum*. He made successful infections of *Pinus sylvestris, Pinus banksiana* and *Pinus rigida* with true aeciospores from galls on *P. banksiana* and *P. rigida*. In his cytological studies he finds intercellular hyphae in the cortex and in rays of phloem and xylem. Haustoria are seldom found in young infections but are abundant in old infections. He interprets this difference in

[1] Supplement to article in The Botanical Review 1: 327–354. 1935.

the abundance of haustoria as evidence of a change in nutritional relations. He groups the host-plant reactions under three types: defoliation without permanent injury, death of leaders, and formation of galls. Comparing tissue from the first and second stages he found that in the first the rust mycelium had been cut off and checked by a cork layer, while in the second the mycelium had penetrated through the cork tissue into other living tissue where it remained alive for a time. It was this persisting mycelium which produced many haustoria. McKenzie concluded that the rust had not succeeded in attaining mutualistic relations with the host and that the transient infection indicated resistance of the host (17).

Thatcher made an interesting study of resistant-type flecks in Vernal wheat infected with race 34 of *Puccinia graminis tritici*. He made microscopic examination of the leaves from 18 hours after inoculation to the fifth day. On the fourth day, minute etiolate flecks were visible under a hand lens. The cells making up these flecks were dead or dying and contained degenerate chloroplasts, but they contained also well developed living haustoria. Even on the fifth day the entire mycelium of the fleck seemed alive. Thatcher concludes emphatically "that a fungus secretion leads to changes that cause death of the host cells some time before the parasite is itself injured" (35).

Dorothy Ashworth presents a detailed cytologic study of the life history of *Endophyllum sempervivi*. This history gives a picture of mutualism in a perennial rust. Spring infections by basidiospores into epidermal cells of leaves of *Sempervivum* become intercellular and haustorial, but, instead of producing spores, the mycelium grows down through the leaves into the cortex of the underground stem. It perennates here, but, since it does not during the first season approach the growing tip, new leaves at the centre of the rosette are free from disease to the end of that season. Renewed activity of the mycelium coincides, the next spring, with the growing season of the host. The mycelium now passes toward the growing point and into new leaves where sporulation follows. The mycelium in the infected leaves is thin-walled and has few haustoria. It shows marked growth in the direction of the long axis of the leaf but masses in the sub-stomatal spaces for sporulation. The mycelium of the underground stem is characterized by irregular form and by numerous stout haustoria (2).

This life history makes an interesting comparison with that of *Aecidium punctatum* Pers. to which I referred earlier (24), and *Endophyllum sempervivi* may be added to the company of *Aecidium punctatum* and *Chrysomyxa Pyrolae* as "outstanding examples of restraint on the part of the parasite toward the host in which they perennate" (24). *Aecidium punctatum* Pers. is perennial in roots and rootstocks of *Hepatica acutiloba.* It develops in the spring leaves while they are still in the bud; when they first appear above ground, the leaves are erumpent with spermogonia. These infected leaves develop into stiff reduced blades set on abnormally long petioles. Aecidia quickly follow the spermogonia and develop thickly on both leaf surfaces. By the end of May the infected leaves are dead. This result of heavy sporulation is in sharp contrast to the long life of uninfected leaves. The latter are active throughout the summer, supplying material for the over-wintering buds, and they themselves over-winter. In the rusted plants the photosynthetic work is carried out by a second crop of leaves in which the rust does not develop. Like the rust in the underground stem of *Sempervivum,* that in the underground parts of *Hepatica* remains dormant until the following spring. Thus an infected *Hepatica* plant in May will have three sets of leaves: reddened, overwintered, uninfected, lower leaves; infected, early spring leaves which, because of their hypertrophy, stand highest; and the tender, green, uninfected second-crop leaves of the spring. Rust-infected *Hepatica* plants have persisted for years in my woodland garden in vigorous condition, although with reduced blooming. The perennial mycelium in the rootstock occurs in cortex, in bundle sheath and in both ray cells and vessels of the xylem. In addition, it occurs in cortex, sheath and vessels of the wiry adventitious roots. In the roots haustoria are scarce, but intracellular hyphae penetrate the vessels and the cells of the bundle sheath. They often fill the cells of the bundle sheath, with branching runners, penetrating from cell to cell for long distances. The hyphal cells are larger in diameter and thicker walled than those of the leaves. In the rootstock the intercellular hyphae are frequently heavily encased in a brown-staining substance which is distinguishable from the host-cell wall. We note that Thatcher, investigating Kubanka wheat infected with *Puccinia graminis tritici,* concluded from microchemical tests that the enveloping material of the encysted haus-

toria was of fungal origin (35). The haustoria of the rootstock in *Hepatica* are also more or less heavily encased and range from small buttons completely encased to huge convoluted knots with lobes projecting from the sheath. These sheathed haustoria make a striking contrast to the numerous thin-walled haustoria in the *Hepatica* leaves where there is every indication of healthy growth of the rust in a healthy host cell. In the *Hepatica* leaf the rust practices a restrained feeding which breaks down only at the incidence of sporulation; in the rootstock there is evidence of lack of adjustment, but the rust makes luxuriant growth and is able to persist and grow into new tissues coincident with the awakening of the host plant to renewed activity in the spring. This again is like the habit of *Endophyllum sempervivi.*

An instance of heavy encasement attendant upon unsuccessful growth of a fungus is given by Bache-Wiig for the bracken parasite, *Cryptomycina Pteridis.* Where conidia had failed to penetrate epidermal cell walls the cell wall had become thick and dark in color below the appressorium. Even after penetration into the epidermal cell the fungus is, at times, confined to the single cell by a thick dark sheath formed around it (3).

A. M. Brown has made observations upon the perennial rust, *Puccinia minussensis* on *Lactuca pulchella.* He reports that "The perennial mycelium seems to alternate from the binucleate to the uninucleate condition and vice versa, depending apparently on the maturity of the host tissue involved and the food supply available to the fungus" (4).

Color changes in the leaves of infected plants seem evidences of altered metabolic reactions in the host cells and, when extreme, may be interpreted in terms of resistance. The following reports are given in order of increasing severity of the disturbance. Ruehle, reporting *Uredo sapotae* for the first time in the United States, notes that the fungus produces red-purple spots on leaves of *Sapodilla* but does not cause serious defoliation (27). Presley and King, describing *Puccinia stakmanii* n. sp. on cotton, note that anthocyan is produced by the leaf around the pycnial cluster (23). Arora, in a study of some rusts of Allahabad, notes that the mycelia caused disappearance of chlorophyll and disorganization of the host cells (1). An extreme case outside the rust group is reported by McDonough for *Sclerospora graminicola* on seedlings of *Selaria.*

The parasite early became systemic in seedlings. It resulted in stunting of the seedlings and in necroses of cells not even in contact with hyphae (16).

Hart and Allison were the first to report upon the browning reaction of Kubanka wheat when infected, at high temperatures, by certain races of *Puccinia graminis tritici.* This was not only a reaction of the host tissues, but seemed due, at least partly, to an encystment by a brown cell-wall-like deposit of many of the rust haustoria. Hart and Allison thought that the structures represented attempts at spore formation (11). Thatcher is more interested in the browning of Kubanka wheat as evidence of a change from susceptibility to resistance on the part of the host. In an exhaustive series of studies of osmotic and permeability relations in parasitism, Thatcher uses changes in host-cell permeability as an index of degrees of parasitism, since it indicates degrees of successful feeding by the parasite. In all cases, both for rusts and for a variety of other fungi, he found that the osmotic pressure of the cells of the parasite where higher than that of their respective host cells and that permeability increase is an effect of tissue invasion by a fungus in its respective host. For example, he found that *Puccinia graminis tritici,* race 21, caused an increase in permeability to cells of the susceptible wheat varieties, Mindum and Little Club, while resistance of Mindum wheat to race 36 was associated with a local decrease of host-cell permeability (34). Thatcher applied his microchemical tests for permeability to successive stages in the browning of Kubanka wheat and concluded that the browning "is an expression of a change in the micro-environment of the fungus induced essentially by high temperature which so modifies the interaction between host and parasite that the host reaction changes from complete susceptibility to a type of resistance leading to a cessation of fungal growth". By this and five other microchemical examinations of infected cereal tissues Thatcher concludes that permeability decrease is one factor tending to confer resistance; and that the delicacy with which the determining factors are balanced seems to indicate that some physiological factor such as a fungus enzyme may effect changes in the host-cell membrane (35).

Humphrey and Dufrenoy are also attacking the problem of host-parasite relations by means of cytochemical reactions. From their

microchemical tests upon leaves of oat plants infected with crown rust they believe that extremes of response to infection as well as all intermediate types of reaction can be interpreted cytochemically. From the living cells of a susceptible host, they state, the invading hyphae draw phosphorus compounds because of increased cell permeability; there is a secretion of vacuolar material out into the intercellular spaces, and phenolic materials are collected within the vacuoles into spherical bodies. In the resistant oats localized necrotic areas develop, and the mycelium, failing to make contact with living cells, induces a breakdown of nucleoproteins. The phenolic compounds yield deep-colored quinoid derivatives which "fix" constituents of the tissues around the infection area (13).

Alteration of cell contents in infected tissues has been reported for telial galls of *Gymnosporangium juniperi-virginianae*. Smits and Peterson finds a much higher concentration of carotene in the galls than in the leaves of the host. They state that the gamma isomer of carotene, rare in plants as far as they know, has not been found in as great concentration from other sources as in galls (29).

A variety of cultural experiments add data upon susceptibility and resistance. Gottlieb and Hart report negative results in testing the reaction to water-soluble growth substances of several varieties of wheat and oats under infection by physiological races of *Puccinia graminis tritici* and *Puccinia graminis avenae*. They conclude that water-soluble growth substances do not play an important role in the problem of resistance in cereal plants and that pathological differences in physiological strains of rust species may not be attributed to different growth substances (9).

Susceptibility with reference to the age of the host has been tested for *Cronartium ribicola*. Pierson and Buchanan, working with seedling of *Pinus strobus,* found that needles of the current season are low in susceptibility, needles of the second and third season are higher in susceptibility, and needles of the fourth season are of intermediate susceptibility. This ratio corresponds to the canker development, for the majority of cankers form on internodes of the second and third seasons (22).

Newton and Johnson tested the effects of temperature and light on the reactions to leaf rust, *Puccinia triticina* Erikss., of varieties

of wheat. They report that with a large number of races both temperature and light exercise a marked influence, with the more marked changes produced by variation of temperature (21).

Yarwood and Childs, testing the effects of rust infection on the dry weight of host tissues, found in ten out of eleven different rusts that the local dry weight per unit area was greater in rusted than in healthy tissue. In their summary we have a picture of the metabolism of a rust parasite. Rust infection apparently decreases the translocation of food from infected leaves because it interferes with photosynthesis; therefore less food is formed. It increases the respiration of infected tissues; therefore carbohydrates are used up. It causes fixation of dry weight in the infected tissue, as the rust fungi seem to be nourished principally by the labile organic materials of photosynthesis (37).

In this same connection comes a study by H. C. Murphy upon the effect of crown rust on the composition of oats. Sugars were noticably decreased in infected plants. Murphy explains this as due not only to the use of the photosynthate by the rust but to interference with the act of photosynthesis by broken epidermis, obstruction of the flow of liquids, chlorosis and necrosis, and shading of the host cells (19).

If, as I have suggested, "specialization is a corollary to obligateness" (24), the continuing reports of the determination of new physiologic races of rusts is the conclusive evidence of their adaptive powers. *Gymnosporangium globosum* Farl. might stand as an example of little race specialization, since MacLachlan reports it as occurring on at least ten genera of Pomoideae and three species of *Juniperus* (15). Yet if economic demands should push the work perhaps many physiologic races of *Gymnosporangium globosum* Farl. might be isolated.

In the heteroecious rusts of *Uredinopsis* Faull reports less specialization in the case of aecidial hosts than in the telial hosts. There is incidental evidence, he writes, that most species of *Abies* are more or less susceptible to all species of *Uredinopsis,* and all species of *Uredinopsis* may be passed to most species of *Abies,* while the species of *Uredinopsis* are closely restricted to their fern hosts (6).

Work done with the pine blister rust removes the ban from certain varieties of red currants. Hahn has demonstrated the im-

munity of the old varieties, Red Dutch and Viking, as against the common susceptible *Ribes sativum* (10). Snell reports that red currant varieties cannot be considered dangerous to white pine (30). Mielke reminds us of the shifting nature of resistance reactions by demonstrating that, although *Ribes lacustre* has lower susceptibility and lower telial production than three other species of *Ribes,* it is capable of rapidly intensifying blister rust on western white pine (18).

Puccinia asparagi offers another illustration of shifting resistance reactions. Its excessive virulence for asparagus crops has been held in check in recent years by the use of the resistant Martha and Mary Washington varieties of *Asparagus.* However, Fulton reminds us that the Washington varieties of *Asparagus* are resistant but not immune to *Puccinia asparagi* and reports that *Puccinia asparagi* is still doing considerable damage in Illinois; that it reached a peak of severity in 1940 to 1941. He relates this to an unusual abundance of inoculum, favorable environmental conditions and the absence of the rust parasite, *Darluca filum* (7).

Crown rust has come in for increasing notice in recent years. Murphy *et al.* report that the reduction of oat yields in Iowa from crown rust, in the period from 1919 to 1938, was more than twice that caused by the stem rust *Puccinia graminis avenae* Eriks. & Henn. This difference is laid to earlier work in distributing oat varieties resistant to stem rust and emphasizes the importance of breeding for resistance to crown rust (20). Rosen and Weetman report that with fall plantings of oats the varieties susceptible to and infected by crown rust gave a reduction in grain yields and sustained more severe frost injury than resistant varieties (26). Rosen also reports over-wintering of the uredinial stage of crown rust of oats in Arkansas in 1941–1942, but has not determined whether mycelium or uredinial primordia were responsible (25). Buchholtz reports that the 1941 epiphytotics of *Puccinia rubigovera* and *Puccinia coronata* in South Dakota were the worst known thus far in eastern South Dakota (5).

Much work has been done also with stem rust of oats. Levine and Smith consider the physiologic specialization of *Puccinia graminis avenae* in the United States as restricted, but in a comparative study of the reaction of seedling and maturing stages of oats to the rust they inoculated 10 physiologic races of the rust on

27 varieties of oats. They concluded that the seedling reaction is a reliable index of the reaction of the adult oat plants to the races of the rust (14). Tervet and Hart report a variation in the reaction of Anthony oats to race 5 of *Puccinia graminis avenae*. They point out that selection within a variety is an excellent means of obtaining rust-resistant strains and that probably many disease-resistant crop plants need periodic re-examination, exposure to artificial epidemics, and re-selection within the variety to ensure the maintenance of resistance (33). Stakman and Loegering point to race 8 as a possible danger to resistant oat races of Victoria × Richland crosses. They conclude: "It is clear that there have been decided and important changes in the prevalence of races of *Puccinia graminis tritici* and equally great changes may or may not occur in *Puccinia graminis avenae*" (31). This reminds one of the reference by Thatcher to the delicacy with which the determining factors are balanced between the diverse manifestation of resistance and susceptibility to rusts (35).

The work with *Puccinia graminis tritici* in breeding for rust resistance still leads, since here one is dealing with danger to the staff of life. In 1935 I quoted Humphrey who gave the total number of physiologic races of *Puccinia graminis tritici* to date as "something like 152 (24). In 1942 Stakman *et al.*, in announcing an unusually virulent race of wheat stem rust collected from Peru, numbered it 189 in the Stakman and Levine key. A quotation from their report brings us back again to the problem of the nature of resistance and susceptibility. "The existence of so virulent a race as 189 is definite confirmation of the statement so often made in the past that nature can produce very virulent races of rust just as man can produce very valuable varieties of wheat". . . . "Therefore it is important to study the prevalence and distribution of physiologic races, eradicate the alternate host when feasible and continue breeding work to find and combine genes for resistance in the most effective manner possible". Again, Stakman emphasizes variability: "A variety of wheat may be resistant in one region and susceptible in another in the same year. A variety may behave differently in the same place in different years. Changes may be gradual or sudden. There is little definite information regarding causes for changes in previous years although seasonal temperatures, distribution of wheat varieties and conditions affecting winter survival of the fungus are pertinent" (32).

The importance of breeding for rust resistance is well illustrated by the history of work with bean rust. Wingard's pioneer experiments in the development of rust-resistant beans by hybridization (36) turned attention to this rust. Harter and Zaumener state that in 1935 two races of bean rust had been differentiated. Now they have announced the differentiation of 20 physiologic races of *Uromyces phasioli typica*. These were established on the basis of the size of a pustule at the end of 14 days on seven different host varieties from the United States and Hawaii (12).

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Cytotaxonomy of Nicotiana	T. H. GOODSPEED *University of California*
Tissue Responses to Physiologically Active Substances	BETTY F. THOMSON *Connecticut College*
Absorption of Water by Plants	PAUL J. KRAMER *Duke University*
The Proper Designation of the Vascular Plants...	THEODOR JUST *University of Notre Dame*
Bacterial Galls	A. J. RIKER *University of Wisconsin*
Control of Fungi in Lumber during Air-Seasoning	A. F. VERRALL *U. S. Department of Agriculture*
Detached Leaf Culture	C. E. YARWOOD *University of California*
Laboratory Testing of Spraying and Dusting Fungicides	J. G. HORSFALL *Connecticut Agriculture Experiment Station*
Root Diseases of Deciduous Fruit Trees	J. S. COOLEY *U. S. Department of Agriculture*
Specialization, Hybridization and Mutation in the Cereal Rusts	T. JOHNSON and MARGARET NEWTON *Dominion Laboratory of Plant Pathology*
Preventing Plant Disease Introduction	W. A. MCCUBBIN *U. S. Bureau of Entomology and Plant Quarantine*
Aerobiology in Relation to Plant Disease	E. C. STAKMAN and CLYDE M. CHRISTENSEN *University of Minnesota*

Articles arranged for most recently

Heart Rots in Living Trees	R. W. DAVIDSON *U. S. Bureau of Plant Industry*
Cell Walls	T. KEER *North Carolina State College*

Articles in course of preparation

The Cytology of Fertilization in Angiosperms	L. E. ANDERSON *Duke University*
Development of the Madro-Tertiary Flora	D. I. AXELROD *University of California*
Relation of Wood Anatomy to Taxonomy	I. W. BAILEY *Harvard University*
Rubber Formation in Plants	JAMES BONNER and A. W. GALSTON *California Institute of Technology*
Role of the Endosperm in Seed Development	R. A. BRINK and D. C. COOPER *University of Wisconsin*
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